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DISTRIBUTION OF ZINC AND COPPER IN SEEDLINGS OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.) IN SOLUTION CULTURE

By D. S. RICEMAN* and G. B. JONES*

(*Manuscript received June* 27, 1956)

Summary

Changes in the distribution of zinc, copper, and dry matter in seedlings of *Trifolium subterraneum* L. var. Bacchus Marsh grown in solution cultures which were supplied with copper but not with zinc have been traced during the first 40 days after germination. Increase in total dry weight was accompanied by a rapid decline in the concentration of zinc in the plant parts examined. Symptoms of zinc deficiency were recognizable in the third trifoliate leaf by the time the leaflets opened, 33 days after germination. At that time the concentration of zinc in leaf plus petiole had fallen to 14 p.p.m. in the dry matter. There was a continual net loss of zinc from the cotyledons.

A marked increase in the amount of copper present in roots, and in leaf plus petiole, occurred soon after the addition of copper to the cultures 20 days after germination, but no substantial change was observed in the amount of copper present in the cotyledons or in the hypocotyl plus growing point. These latter tissues had previously lost small amounts of copper.

I. INTRODUCTION

An examination has been made of the distribution of zinc and copper and of dry matter in subterranean clover (*Trifolium subterraneum* L. var. Bacchus Marsh) grown in solution cultures. The investigation followed two courses so that a study could be made of: (*a*) the changes in the distribution of these metals in seedlings grown for 40 days after germination in culture solutions to which zinc had not been added; (*b*) the changes taking place from the 16th day after germination to maturity, in plants grown in culture solutions to which zinc had been added in quantities which provided a wide range of concentrations. The first part of the investigation is described here.

II. MATERIALS AND METHODS

Seed of *T. subterraneum* (var. Bacchus Marsh), which had been sieved to select seed of closely uniform size, was treated to determine the following.

(*a*) *Weight per Seed*

Mean air-dry and oven-dry weight per seed were determined on a sample of 400 untreated seeds.

* Division of Biochemistry and General Nutrition, C.S.I.R.O., University of Adelaide.

(b) Zinc and Copper Content of Seed

Two lots of 100 seeds of known air-dry weight were surface-sterilized and acid-washed in the manner described in (*d*) below, and their zinc and copper contents determined. Mean weights of these metals per seed, oven-dry, were calculated.

(c) Distribution of Dry Weight, Zinc, and Copper between Testa, and Cotyledon plus Embryo

Five grams of seed were surface-sterilized and acid-washed, and then, to soften the testas, transferred to a Pyrex glass dish with 6 ml glass-distilled water and kept in darkness. An additional 1 ml water was necessary after 24 hr and again after a further 48 hr. The water was no more than could be imbibed in a short time. On the fourth day (3 days after germination began, and designated day 3), 309 seeds were removed and divided into testa, and cotyledon plus embryo, for the estimation of zinc and copper distribution and dry weight.

(d) Dry Weight Increase and Distribution of Zinc and Copper in Seedlings

Fifty grams of seed (approx. 4800 seeds) were surface-sterilized for 3 min and rinsed several times with glass-distilled water; then after washing for 3 min in dilute (approx. 20 per cent.) hydrochloric acid that had been purified by distillation in hard glass apparatus, they were again rinsed several times. The sterilizing liquid was made up of equal volumes of 20 vol. hydrogen peroxide and redistilled 95 per cent. ethyl alcohol. After each of the above steps the liquid was removed by suction through a Buchner filter. The prepared seed was spread thinly over the bottom of a plastic (polyethylene) vessel, moistened with 60 ml glass-distilled water, and covered with a sheet of glass. On the following day the swollen seed was distributed on acid-washed, heavily waxed netting over a series of Pyrex glass dishes filled to within 0.5 cm of the top with dilute culture solution. Acid-washed filter paper was rested on top of the seed and covered with a sheet of glass to keep the filter paper in firm contact with the seed. The filter paper was kept moist by a series of filter paper wicks extending down into the solution. The vessels were kept in darkness in the laboratory. Germination was practically complete by the next day, May 27, 1954, and the majority of the radicles were in contact with the solution. This date is designated day 1. On day 2, the few ungerminated seeds and malformed seedlings were removed, after which the filter papers were replaced, but not the glass covers. The vessels were placed in the glass-house on day 5 after the filter papers had been finally removed and the testas gathered and discarded.

The dilute solution used at first in the seedling cultures was made up of: 0.5 ml M ammonium dihydrogen phosphate, 2.5 ml M potassium nitrate, 3 ml M calcium nitrate, 1 ml M magnesium sulphate, and 0.25 ml 10 per cent. sodium chloride, per litre. On day 9, 1.1 mg iron/l was added as

ferrous tartrate (0.5 per cent. ferrous sulphate and 0.4 per cent. tartaric acid, after Arnon (1938)). Fresh solution of the same composition, including 1.1 mg iron/l, was supplied on day 12. On day 20, this solution was replaced by one which contained twice the concentration of major nutrients and sodium chloride, and to which was added 1.1 mg iron and 1 ml of a solution which contained 1 mg manganese, 0.06 mg copper, 0.6 mg boron, and 0.05 mg molybdenum, per litre. No zinc was added. A supplementary addition was made according to plan on day 30, consisting of 4 ml of a solution of M ammonium nitrate and 1.25M potassium nitrate, per litre of culture.

All vessels were washed with dilute (approx. 20 per cent.) distilled hydrochloric acid and water before use. The water used for all purposes was double-distilled, a Pyrex still and condenser being used for the second distillation. The salts used had been treated for the removal of traces of zinc and copper impurity for an earlier series of experiments. Subsequently, however, it became evident that the solutions had become slightly contaminated during the period of storage in polyethylene bottles. The use of polyethylene vessels for this purpose has been discontinued.

The details given below refer to the seedlings removed from the cultures for the estimation of dry weight and of zinc and copper distribution.

Day	No. Seedlings Taken	Growth Stage
9	400	Primary leaf just emerging
16	400	First trifoliate leaf emerging
22	282	Second trifoliate leaf emerging
29	280	Third trifoliate leaf emerging
34	106	Fourth trifoliate leaf emerging
40	108	Fourth trifoliate leaf opening

The seedlings taken on day 9 were divided into: (*a*) cotyledons and their petioles; (*b*) hypocotyl, growing point, and emerging primary leaf; (*c*) root. Those taken subsequently were divided into: (*a*) cotyledons and their petioles; (*b*) primary and trifoliate leaves and their petioles; (*c*) hypocotyl and growing point; (*d*) root. The hypocotyls and roots were rinsed in glass-distilled water before being separated.

In addition to the seedlings taken on day 16, 352 were removed and transplanted directly into culture vessels for the second part of the investigation, which is to be described elsewhere.

For the determination of dry weight the whole samples were placed in weighing bottles and dried in an oven at 90°C for 24 hr; during the first 3 hr the moist air was withdrawn from the oven by means of a suction pump. The dried samples were cooled in a desiccator, weighed, and then transferred quantitatively to digestion flasks and digested with nitric, sulphuric, and perchloric acids. Zinc and copper were extracted

with dithizone and chloroform, and the two metals were estimated simultaneously by means of a polarograph. Details of the procedure have been described by Jones (1952).

III. Results and Discussion

(*a*) *Dry Weight*

The progressive changes in the mean dry weight per seedling are shown in Figure 1. The loss of weight of embryo, cotyledons, and testa during the first 3 days from 9.56 to 8.20 mg (i.e. a loss of 14 per cent.)

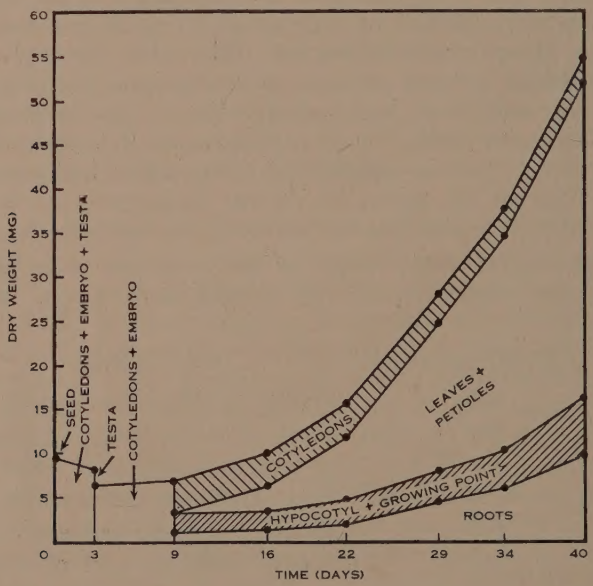

Fig. 1.—Dry weights of plant parts (mg per seedling).

may be ascribed to respiration (McLuckie and McKee 1954). During the next 37 days an eightfold increase in dry weight of seedling (minus testa) took place. The dry weight of the testas on day 3 comprised 21 per cent. of the total dry weight of the germinating seed.

The dry weight of the cotyledons diminished continually, as observed by Black (1955) : on day 40 the mean dry weight of the two cotyledons and their petioles had fallen to 2.87 mg, or 75 per cent. of their weight on day 9. Total dry-weight distribution on day 40 was as follows: cotyledons and their petioles, 5 per cent.; primary leaf, trifoliate leaves, and petioles, 65 per cent.; hypocotyl and growing point, 12 per cent.; roots, 18 per cent.

Evidence of zinc deficiency in the seedlings became clear on day 33, by the time the mean dry weight per seedling had reached approx. 35 mg. The emerging third trifoliate leaf was smaller and more translucent than

earlier leaves had been at a comparable stage; its leaflets unfolded more slowly, and the distinctive tinge of a yellowish green chlorosis became apparent at the base of the leaflets. On day 40 the emerging fourth leaf was affected in this manner, while in the third leaf grey or light brown, shrunken spots had developed each side of the midrib near the base of the leaflets, and some of these shrunken spots had coalesced to form extensive necrotic areas. The leaflets were yellowish green in the centre, but their margins remained a normal green colour. In the third leaf of some of the seedlings a mottled purplish brown zone became discernible between the chlorotic area and the green margin. Leaves affected in this way are illustrated in Plate 1, Figure 1. The first and second trifoliate leaves were apparently unaffected at this stage. The cultures were discontinued before the plants progressed to the "little leaf" stage.

These early symptoms correspond with descriptions of zinc deficiency induced in solution culture by Powrie (1951), and Millikan (1953*a*, 1953*b*). In addition, however, some of the older leaves assumed a "goblet" shape owing to a slight upward curvature, not only of the whole leaflet but of the lateral margins as well. This habit, illustrated in Plate 1, Figures 2 and 3, has been observed in other zinc-deficient cultures, not only in seedlings that have been maintained in zinc-free media, but also when the onset of the deficiency has been delayed for varying lengths of time by the addition of suboptimal quantities of zinc to the culture solution (Riceman and Jones, unpublished data).

(*b*) *Distribution and Concentration of Zinc and Copper*

Changes in the distribution and concentration of zinc and copper are shown in Figures 2-5.

The germinating seed and the developing seedlings gained both zinc and copper from the small traces present as impurity in the salts used for the culture solution. Although the amounts gained were extremely small they represented a considerable proportion of the total weight of zinc and copper per seedling. The early gain in copper was of no consequence, and copper was included among the heavy metals added on day 20. The gain in zinc was sufficient to delay the onset of zinc deficiency by an estimated period of nearly a week.

The selection of seedlings on day 34 from populations of slightly greater density probably accounts for the aberrant, lower mean weights of zinc and copper per seedling at that stage.

(i) *Zinc.*—The original seeds contained a mean of 0.36 μg zinc per seed. On day 3 the amount had increased to 0.46 μg. Of this, 0.11 μg, or 25 per cent., was located in the testa and was no longer available to the developing seedling, which at that stage contained 0.35 μg zinc.

As growth proceeded there was little change in the amount of zinc present in the hypocotyl and growing point, or in the root, but it is

apparent that there was a rapid transport of zinc out of the cotyledons and into the developing leaves and petioles, which also accumulated the bulk of the zinc which found its way into the seedling from external sources. On day 40 the amount of zinc in the cotyledons and their petioles was only 17 per cent. of the amount present there on day 9.

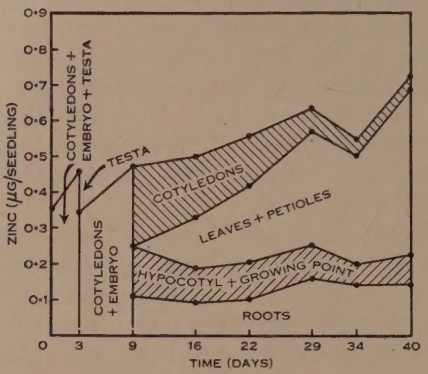

Fig. 2

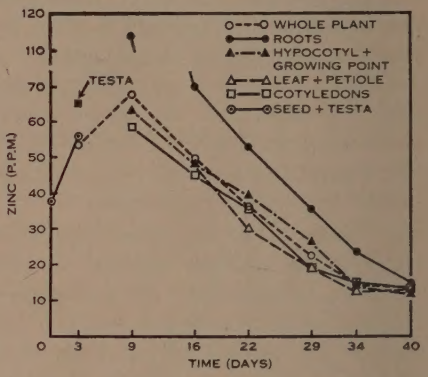

Fig. 3

Fig. 2.—Amounts of zinc in plant parts.

Fig. 3.—Concentrations of zinc in the dry matter of plant parts.

The final distribution of zinc, on day 40, was as follows: 5 per cent. in cotyledons and their petioles; 64 per cent. in primary leaf, trifoliate leaf, and petioles; 11 per cent. in hypocotyl and growing point; 20 per cent. in roots. This was almost identical with the final distribution of dry weight, and the result is to be seen in the uniform final concentration of zinc in all parts of the seedling, namely, 12.4-14.9 p.p.m., as shown in Figure 3.

The concentration of zinc in the whole, oven-dried seed was 37.6 p.p.m. Through the combined influences of the initial loss in dry weight and the small gain in zinc from outside sources, the concentration on day 3 in the whole seed had risen sharply to 55.9 p.p.m. Cotyledons plus embryo, without the testa, contained 53.4 p.p.m. A further rise in concentration in the seedling (less the testa) took place during the interval to day 9. As growth proceeded further the concentration in all parts of the seedling began to fall rapidly to a minimum of 12.4-14.9 p.p.m., the minimum being reached in leaf plus petiole by day 34, and in the remaining parts by day 40. Only the roots contained an appreciably higher initial concentration of zinc (114 p.p.m.) than the other parts of the plant, and this difference was transient.

The rate of loss of zinc by the cotyledons was relatively greater than the loss in dry weight. On the other hand, the rate of gain in dry weight by leaves plus petioles, and by roots, was relatively greater than the gain

in zinc. The hypocotyl plus growing point gained in dry weight while losing zinc.

Zinc deficiency symptoms became clearly visible in the third trifoliate leaf only when the leaflets began to open, on day 33. It is probable that before that time, the concentration of zinc had become sufficiently low to adversely affect the metabolism of the new leaf tissue and so to initiate the development of symptoms. By day 33 the concentration of zinc in leaf plus petiole had fallen to 14 p.p.m. However, only 4 days previously (day 29) when the third leaf had begun to emerge, the concentration in leaf plus petiole had been 19.0 p.p.m. Thus there was a rather precipitate fall of 1.3 p.p.m. zinc/day during this period. Owing to the rapidity of this change in concentration an estimate of the critical concentration of zinc cannot be made, but it may be implied that metabolism in the young leaf became deranged while the zinc concentration of leaf plus petiole of the seedlings was still somewhat in excess of 14 p.p.m. Ozanne (1955) observed evidence of zinc deficiency in young plants of the Dwalganup variety of subterranean clover when concentrations of zinc in the leaves were of the order of 20 p.p.m.

If zinc from sources external to the seed had been entirely excluded from the cultures, seedlings which contained 0.35 μg zinc on day 3 should have reached a dry weight of only 21.6 mg by the time the zinc concentration in the whole plant had fallen to 16 p.p.m., which was the concentration in seedlings in which deficiency symptoms had already become apparent. At a rate of growth comparable with that observed, this point would have been reached within 26 days of germination, or approximately 7 days earlier than it was actually reached in the cultures. Under the same conditions, metabolism would have become deranged before the seedlings reached 18 mg dry weight, and within 23 days of germination, even if the critical concentration of zinc were not reached until the concentration in the whole plant had fallen to as little as 20 p.p.m.

There was no immediate reduction in the rate of dry matter production coincident with the onset of zinc deficiency symptoms, but it seems likely that the observed rate would have been exceeded if zinc deficiency had not supervened. Emergence of the fifth leaf was delayed, and soon after day 40, lesions became extensive and growth practically ceased.

(ii) *Copper.*—The original seeds contained a mean of 0.040 μg copper per seed. On day 3 the whole seed contained 0.055 μg copper, of which 10 per cent. was located in the testa. On day 9, the distribution between cotyledons, hypocotyl plus growing point, and root was approximately equal, although the concentration was much higher in the root than in the rest of the seedling.

The sharp rise observed in the amount of copper per seedling between days 16 and 22 probably took place during the last 2 days of this period,

since copper was not added to the culture until day 20. While the bulk of the additional copper present in the seedling was located in the roots on day 22, a threefold increase in the amount of copper in leaf plus petiole took place during the next 7 days. No substantial change took place in the amount of copper present in the hypocotyl plus growing point, nor in the cotyledons, during this period. These tissues had previously lost small amounts of copper.

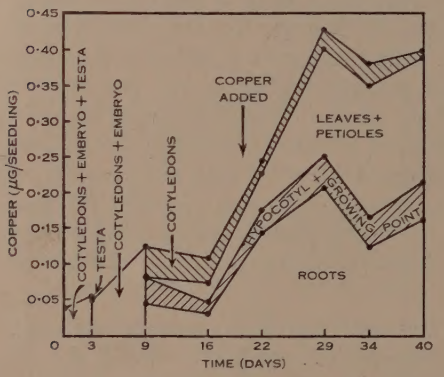

Fig. 4

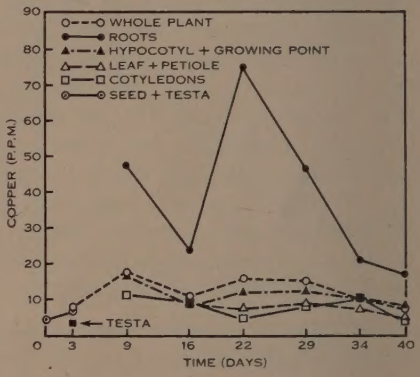

Fig. 5

Fig. 4.—Amounts of copper in plant parts.

Fig. 5.—Concentrations of copper in the dry matter of plant parts.

The final distribution of copper in the seedlings on day 40 was as follows: 3 per cent. in cotyledons and their petioles; 44 per cent. in primary leaf, trifoliate leaves, and their petioles; 13 per cent. in hypocotyl plus growing point; 40 per cent. in root. The amount of copper which remained in the cotyledons and their petioles on day 40 was 23 per cent. of that present on day 9.

The concentration of copper in the original, oven-dried seed was 4.2 p.p.m. On day 3 the concentration in the separated testas was 3.3 p.p.m., in the cotyledons plus embryo 7.7 p.p.m., and in the whole, germinating seed 6.8 p.p.m. Copper was present in much higher concentration in the root than in other parts of the seedling, even before copper was supplied in the culture. The addition of copper to the culture solution on day 20 only temporarily increased the concentration of copper in the various plant parts. The fall in concentration in the roots was resumed immediately, while it was delayed until after day 29 in hypocotyl plus growing point and in leaf plus petiole, and until after day 34 in cotyledons.

IV. Acknowledgments

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ZINC AND COPPER IN SUBTERRANEAN CLOVER

to acknowledge the careful assistance rendered by Miss I. M. Perrott in raising the seedlings, and by Mr. J. S. Charnock, Mr. J. O. McKenzie, and Mr. W. S. Osborne-White in the chemical work.

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Explanation of Plate 1

Fig. 1.—Leaves of zinc-deficient *T. subterraneum* showing the purplish brown zone which separates the expanding chlorotic area from the green margin of the leaflets. In the right-hand leaf a grey, necrotic lesion is to be seen beside the midrib.

Figs. 2 and 3.—"Goblet" habit in leaves of *T. subterraneum*.

INVESTIGATIONS ON BROWN ROT OF APRICOTS CAUSED BY *SCLEROTINIA FRUCTICOLA* (WINT.) REHM.

I. THE OCCURRENCE OF LATENT INFECTION IN FRUIT

By G. C. WADE*

(*Manuscript received April* 23, 1956)

Summary

Evidence is presented that infection of apricot fruit with *Sclerotinia fructicola* may occur early in the fruit's development but remain latent until ripening commences. Evidence from culturing from green fruit is supported by the results of spraying experiments with fungicides and by inoculation experiments. Latent infection is confined to the epidermis and is not associated with any particular position on the fruit surface. Histological studies indicate that infection takes place through the stomata. It is tentatively suggested that an inhibitory substance is present in green fruit, and maintains infections in a latent condition. The presence of a substance inhibitory towards a species of *Histoplasma* is demonstrated. This substance begins to disappear as ripening commences.

I. INTRODUCTION

This paper is the first of a series reporting the results of investigations on brown rot of apricots conducted in Tasmania. *Sclerotinia fructicola* is the only species associated with the disease in the State. In many years heavy losses, often exceeding 50 per cent. of the total crop of some growers, are experienced.

In earlier papers (Wade 1951, 1952) the suggestion was made that latent infection with *S. fructicola* occurred early in the development of the fruit, but that these infections did not develop and cause rotting of the tissues until the fruit became mature. The evidence for that suggestion was obtained from three experiments on the relation of fruit maturity to susceptibility to brown rot. In each of those experiments cultures from a proportion of surface-sterilized unripe fruit, taken at intervals from the same trees, developed *S. fructicola* in culture. Support for the hypothesis was obtained from experiments on control by spraying with fungicides. In those experiments significant control was not obtained with cover sprays applied close to picking time unless they had been preceded by sprays at the petal fall or shuck fall stages.

Further evidence for the occurrence of latent infection of fruit is presented in this paper.

II. INCIDENCE OF INFECTION IN GREEN FRUIT

During the seasons from 1952-53 to 1954-55 inclusive, samples of green fruit were taken from trees on five different orchards, surface-sterilized, and plated onto agar. The results of the experiments in the

* Department of Agriculture, Hobart, Tas.

1949-50, 1950-51, and 1951-52 seasons, which were reported previously (Wade 1951, 1952), provided evidence that between 1 and 17 per cent. of fruit were carrying infection. The methods employed, and results obtained in subsequent seasons, are reported here.

Method.—Whole fruit were surface-sterilized by immersion in 1 : 1000 mercuric chloride solution plus 1 : 200 "Emulsit" wetting agent, and then washed in three changes of sterile water. Early in the season whole fruit were plated onto agar, but later samples were taken from the fruit

TABLE 1

INCIDENCE OF INFECTION ON GREEN FRUIT

Figures in brackets represent the number of trees from which the sample was taken. P, pink stage; P.F., petal fall; F.B., full bloom

Season	Orchard	Treatment	Date	Number Sampled	Latent Infection (%)	Infection at Harvest (%)
1952-53	A	Nil	18.xii.1952	80(4)	8	5.1
1952-53	A	Thiram (P.F.)	18.xii.1952	80(4)	1.2	0.9
1953-54	B	Nil	19.x.1953	72(6)	6.9	17.6
1953-54	B	Bordeaux (P) / Thiram (P.F.)	18.x.1953	144(12)	0	5.6
1953-54	B	Nil (P) / Thiram (P.F.)	19.x.1953	72(6)	0	5.8
1953-54	B	Nil (P) / Thiram (F.B.)	19.x.1953	72(6)	0	8.4
1953-54	C	Nil	19.xi.1953	72(6)	11	27.0
	D	Nil	4.xi.1953	72(6)	8.3	*
1954-55	E	Nil	3.xii.1954	48(4)	41	13.7
	E	Nil	20.xii.1954	72(6)	55	18.0

* No record taken.

with a sterile cork-borer 5 mm in diameter. Six samples were taken from each fruit and placed in close proximity. In experiments conducted prior to the 1953-54 season, cultures were made onto potato dextrose agar, but difficulty was experienced with cross-contamination resulting from rapid growth of the fungus on the medium. Subsequently, distilled water agar has been used, and less trouble of this kind has been experienced.

Sampling was random. In 1952-53 20 fruit were taken from each tree, but in subsequent years this was reduced to 12 fruit per tree.

Results.—The results are summarized in Table 1.

In all cases where cultures were made from fruit from untreated trees, *S. fructicola* was isolated. It was only isolated from one fruit out of 360 from trees which had been sprayed with thiram at the petal fall or full bloom stages more than 8 weeks before the date of sampling. This marked influence of previous treatment removes the possibility that the

organism arose in culture from spores which had simply been trapped in the hairs on the skin and had escaped sterilization.

Latent infection represented a high proportion of the infection apparent on the fruit at harvest. The apparent contradiction in the 1954 figures, when the latent infection recorded was higher than that recorded on the fruit at harvest, was explained when apparently sound fruit from the trees were stored at room temperature for 24 hr. During that period over 90 per cent. of the fruit developed brown rot. As weather conditions during the ripening period were unfavourable for infection it is considered that most infection which developed after picking was the result of latent infections.

III. Site of Infection on the Fruit

(*a*) *Depth of Penetration*

In the 1954-55 season an experiment was conducted to determine whether infection occurred in the epidermis or in the flesh of the fruit.

Method.—Twelve fruit were taken at random from a tree which had previously been shown to be heavily infected. The fruit were sterilized as previously described, and cores of tissue removed from eight positions on each fruit with a sterile cork-borer. Each core was then divided into three portions: (*a*) including the epidermis; (*b*) flesh from the centre of the core; (*c*) flesh adjacent to the stone. The material was then plated onto water agar.

Results.—*S. fructicola* was isolated from the epidermis of 10 out of the 12 fruit, and in 26 samples of epidermis out of 96, but not from any of the flesh samples.

(*b*) *Distribution on the Surface of the Fruit*

Experiments were conducted in 1954 to determine if infection was associated with any particular positions on the surface of the fruit.

Method.—Twelve fruit were taken at random from each of four trees in the first experiment and from the same and two additional trees in the second experiment. The fruit were sterilized as previously described and cores taken from eight positions on each fruit with a sterile cork-borer. Positions 1 and 5 were from the stem end, 4 and 8 from the distal end, and 2, 3, 6, and 7 from median positions. The cores were plated out onto water agar.

Results.—The total number of isolations from each position for each tree are shown in Table 2. The maximum possible number of isolations from any one position on fruit from a single tree is 12.

The results were examined statistically and it was concluded that the evidence of these experiments suggests that the distribution of infection is random, and there is no association with any particular positions on the surface.

IV. Inoculation Experiments

In the 1955-56 season inoculation experiments were conducted to determine if latent infection could be induced by artificial inoculation, and the stages when the trees were susceptible to infection.

Method.—Portions of limbs were inoculated by spraying with a spore suspension of *S. fructicola.* The inoculated portions of the limbs were then completely covered with plastic sleeving for 48 hr.

Table 2

DISTRIBUTION OF LATENT INFECTIONS ON THE FRUIT SURFACE

Date	Tree	No. of Isolations at Position								Number of Infected Fruit (÷ 12)
		1	2	3	4	5	6	7	8	
3.xii.1954	A	5	4	4	4	4	2	1	0	8
	B	2	1	1	1	3	2	2	2	6
	C	1	1	1	2	0	0	0	0	3
	D	1	2	0	1	1	1	1	3	7
20.xii.1954	A	1	2	0	2	1	1	2	2	7
	B	2	1	1	0	2	1	1	1	6
	C	5	3	3	2	3	3	2	3	8
	D	1	1	3	1	2	1	2	1	6
	E	1	3	1	2	2	5	2	3	9
	F	0	0	1	0	2	2	0	0	4

The fruit from the inoculated limbs were harvested during the third week of December when the fruit were almost fully developed, but before ripening, as indicated by paling of the ground colour of the fruit, had commenced. The fruit were then surface-sterilized by immersion in a 1 : 1000 solution of mercuric chloride plus 1 : 200 of "Emulsit" wetting agent. They were then washed in three changes of water and placed on a layer of moistened, sterile sand in baking dishes. The dishes were covered with plastic bags, and the fruit were ripened artificially at 25°C. The trays were observed daily for the development of brown rot, infected fruit being removed as soon as they were detected.

In experiment A at Cambridge four trees were used, three limbs being inoculated at each stage on each tree.

In experiment B at Cambridge two trees, different from those in experiment A, were used to determine whether inoculation when the fruit were half-grown could induce latent infection. Six limbs on each tree were inoculated.

In experiment C, conducted on a single tree in Hobart, six limbs were inoculated at each stage.

Results.—The results are shown in Table 3.

Inoculations at full bloom and shuck fall were both effective in inducing latent infection in the fruit, while inoculations at petal fall and when fruit were half grown were not shown to be effective.

TABLE 3

PERCENTAGES OF FRUIT WHICH DEVELOPED APPARENT LATENT INFECTION AFTER INOCULATION

Experiment	Full Bloom	Petal Fall	Shuck Fall	Fruit Half Grown	Uninoculated
Cambridge A	15.6*	6.5	14.9*	—	2.9
Cambridge B	—	—	—	6.0	9.9
Hobart	15.0*	—	17.0*	—	1.6

* Significantly different from control.

V. Evidence from Spraying Experiments

In the course of the spraying experiments it was shown that control is not obtained unless applications are made during the full bloom to "shuck fall" stages. As this aspect of the work will be discussed in detail in a separate paper only brief reference to the relevant results of three representative experiments (see Table 4) is made here.

Experiment C showed that in the season in which the experiment was conducted spraying at full bloom, petal fall, and shuck fall was more effective than at full bloom and petal fall or at petal fall and shuck fall only. It also showed that significant control of brown rot at harvest was obtained when early sprays were not followed by cover sprays.

Experiment A demonstrated that cover sprays, which commenced in December, did not give significant control unless preceded by a spray at petal fall, while in experiment B the use of a shuck fall spray in addition to the petal fall spray greatly improved control.

In November all blighted twigs were removed from all trees in the experiments, so that there was no possibility that the early sprays exerted control by reducing the number of twig infections, which later provided a source of inoculum for other fruit on the tree.

VI. Histological Evidence

Initial histological studies employing paraffin sectioning of fruit tissue at 10 μ failed to provide evidence of latent infection. A number of staining schedules, including Heidenhain's haematoxylin, Stoughton's schedule, Pianese III*b*, and safranin–light green were employed.

A technique was then evolved in which strips of the epidermis were separated, stained, and mounted.

Method.—In order to remove surface hairs, which interfered with the examination of the epidermis, the fruit were shaved with an electric razor. Thin peelings from the fruit were then fixed in formal-acetic-alcohol

and stored in the fixative until required. They were then washed in running water for at least 6 hr, placed in a 1: 1000 solution of pectinase, and held at approximately 37°C until the flesh had softened but the epidermis was unaffected (about 16 hr). The material was then placed, epidermis downwards, under a dissecting microscope, and the softened

TABLE 4

INFLUENCE OF TIME OF FUNGICIDE APPLICATION ON BROWN ROT INCIDENCE

Experiment	Treatment				Percentage Brown Rot
	Full Bloom	Petal Fall	Shuck Fall	Cover Sprays	
A	Nil	Nil	Nil	Nil	20.4
	Thiram	Thiram	Nil	Thiram	10.8
	Nil	Thiram	Nil	Thiram	9.6
	Nil	Nil	Nil	Thiram	17.6
Min. sig. diff.: At 5% level, 7.4 At 1% level, 10.4					
B	Nil	Nil	Nil	Nil	7.2
	Thiram	Thiram	Nil	Thiram	4.8
	Thiram	Thiram	Thiram	Thiram	1.3
Min. sig. diff.: At 5% level, 2.96 At 1% level, 3.97					
C	Nil	Nil	Nil	Nil	13.8
	Thiram	Thiram	Thiram	Thiram	2.6
	Nil	Thiram	Thiram	Thiram	5.2
	Thiram	Thiram	Nil	Thiram	4.8
	Thiram	Thiram	Thiram	Nil	9.0
Min. sig. diff.: At 5% level, 2.0 At 1% level, 2.7					

flesh scraped off with a scalpel. The epidermis was then washed carefully and, using the technique described by White and Baker (1954), gently boiled for 2 min in lactophenol containing 0.1 per cent. cotton blue. The lactophenol was allowed to cool and the material was then mounted in plain lactophenol. To preserve the preparations they were ringed with nail lacquer.

Results.—In preparations from some of the fruit from trees which showed a high percentage of latent infection, conidia of *S. fructicola* were found abundantly on the surface of the epidermis. In order to confirm the identity of the conidia a number were measured with an ocular micrometer. The dimensions and shape of the conidia agreed with those of *S. fructicola*. The majority of the conidia had not germinated, but in a number of instances germination had occurred and hyphal growth had developed on the epidermis, though there was no convincing evidence of penetration directly through the cuticle (Plate 1, Fig. 1). In a few

cases the hyphae grew towards a stoma (Plate 1, Fig. 2) though penetration by hyphae growing from a conidium distant from a stoma was not observed.

In a number of cases, however, conidia were found lying within the stomatal cavity; some of these had germinated and a penetration tube had entered cells surrounding the cavity (Plate 1, Fig. 3). Apparently further growth was arrested, and intercellular penetration to cells up to three away from the point of infection, as described by Adam *et al.* (1949) for *Colletotrichum* infection of oranges, was never observed.

Examination of many preparations failed to provide evidence of appressorium formation, which has been recorded for another species of *Sclerotinia* (de Bary 1886) and is a common feature of many fungi which produce latent infections: e.g. *Phoma citricarpa* (Kiely 1949), *Colletotrichum* on oranges (Adam *et al.* 1949), and *Colletotrichum* and *Gloeosporium* species on tropical fruit (Simmonds 1940).

VII. Possible Role of an Inhibitory Substance

In the course of experiments in which pieces of tissue from green apricots were placed onto potato dextrose agar to determine the amount of latent infection with *S. fructicola* occasional contamination with a fungus, identified by the Commonwealth Mycological Institute as a species of *Histoplasma,* occurred. It was noted that the growth of the fungus close to the tissue was inhibited and clear zones remained which were not invaded by the fungus (Plate 1, Fig. 4). Experiments were then conducted to determine whether an inhibitory effect could be demonstrated towards *S. fructicola,* and whether tissue from green apricots was always inhibitory towards *Histoplasma,* and to determine the activity of different portions of the fruit.

Method.—Fruit were surface-sterilized and a core of tissue removed with a sterile cork-borer and placed on potato dextrose agar seeded with spores of *Histoplasma.* In the majority of experiments the epidermal layer and underlying flesh to a depth of approximately 4 mm was used, but in one experiment one series was conducted in which the epidermal layer was removed. The plates were incubated for 3 days, and the width of inhibition zones measured.

Results.—Summarized results of several representative experiments with *Histoplasma* as test organism are presented in Table 5. No inhibition zones have been demonstrated from fruit after the last date in the table.

These experiments demonstrated that green apricot tissue contains a substance which is inhibitory to the growth of *Histoplasma,* and suggest that the substance occurs chiefly in the flesh near the epidermis, and that it begins to disappear as the fruit approach maturity.

Similar experiments have been conducted using *S. fructicola* as test organism, but clearly defined inhibition zones have not developed, though

a temporary reduction in sporulation around the tissue has been observed in a few instances. Zones of this type developed around tissue from 2 out of 24 fruit in one such experiment.

TABLE 5

INHIBITORY EFFECT OF GREEN APRICOT TISSUE TO *HISTOPLASMA*

Date	Tissue	Number of Samples	Number Showing Inhibitions	No. of Inhibitions of Width (mm)			
				<1	1-1.9	2.0-3.9	>3.9
Nov. 6	Flesh + epidermis Flesh on agar	24	21	1	7	11	2
Nov. 9	Flesh + epidermis Flesh on agar	24	15	0	8	6	1
Nov. 12	Flesh + epidermis Epidermis on agar	36	0	0	0	0	0
Nov. 12	Flesh + epidermis Flesh on agar	36	28	6	9	13	0
Nov. 12	Macerated flesh + epidermis	36	0	0	0	0	0
Nov. 12	Flesh immediately below epidermis	36	31	16	10	4	1
Nov. 12	Flesh near stone	18	0	0	0	0	0
Dec. 22	Flesh + epidermis	12	3	0	3	0	0

Although in the results reported above the samples were taken with a cork-borer, other experiments have been conducted in which irregular-sized samples were cut from surface-sterilized fruit with a sterile scalpel. They were then placed on the agar so that a flat vertical section, including the epidermis, was in uniform contact. With this technique wider inhibition zones of up to 5 mm in width have frequently developed, and the result of such an experiment is illustrated in Plate 1, Figure 4. When this type of section was used, the inhibition zones tended to be wider around the portion of the fruit containing the skin.

VIII. DISCUSSION

The development of *S. fructicola* from cultures of green fruit provides strong evidence that infection of the fruit occurs at an early stage of development, but such infection remains latent until ripening commences. This infection frequently represents a high proportion of the total rot that develops on the fruit at harvest and in storage. Morschel (1955) found similar evidence of latent infection of green fruit.

The application of a fungicide at the petal fall stage reduces the incidence of latent infection considerably, and cultures from green fruit from trees which had received this treatment rarely developed the organism. It was further found that significant control of the disease at harvest may not be obtained unless this spray is applied.

In one season an improvement in control was achieved when an additional spray at shuck fall was applied, and an additional spray at full bloom improved it in another season.

The exact period during which latent infection may be initiated has not been determined, but the present information suggests that it occurs chiefly between the full bloom and shuck fall stages. Earlier infections usually result in general invasion of the flower, resulting in its complete destruction so that no fruit is set. In experiments conducted in the Murrumbidgee Irrigation Area Morschel (1955) found that latent infection could be induced by inoculations at the flowering stage and, to a less extent, at petal fall.

Inoculation experiments described in this paper showed that latent infection may be initiated by inoculations at full bloom and at shuck fall, but not when the fruit are half grown. The failure of inoculations of half-grown fruit to produce infection leading to the development of brown rot in ripening fruit would appear to discount the possibility that spores are simply trapped in the epidermal hairs, and germinate and produce infection as the fruit ripen.

Evidence from culturing showed that latent infection is confined to the epidermis, and the fungus has not been isolated from the flesh of immature fruit. Histological studies confirmed this conclusion, and no fungal hyphae have been found in the flesh of several hundred sections examined. These studies suggest that the usual mode of entry is through the stomata, and direct penetration through the cuticle has not been observed. In studies on the method of penetration of *S. fructicola* into mature fruit, Curtis (1928) found that usual methods in apricots were through stomata or directly through the cuticle.

Cuticular penetration, with the establishment of hyphae immediately below the cuticle, has been described for many other fungi which produce latent infection. Simmonds (1940) found it common to infection by species of *Gloeosporium* and *Colletotrichum* on banana, papaw, and mango, and Adam *et al.* (1949) described similar features of the infection of orange by *Colletotrichum gloeosporioides*. Simmonds considered that if stomatal penetration by the fungi he studied occurs at all, it is of secondary importance. Establishment of latent infections by stomatal penetration was described for *Botrosphaeria ribis* on avocado by Horne and Palmer (1935). Their account refers to the development of hyphal masses in the stomatal cavity, which were not observed with *S. fructicola*. A rather similar type of penetration through the lenticels was described by Kidd and Beaumont (1925) for a number of fungi infecting apple fruit. In the studies on *S. fructicola* no evidence was found that latent infection advances beyond the stage of penetration of a hypha into a cell bordering the stomatal cavity. In many cases ungerminated conidia were found

within the stomatal cavity. It appears likely that these conidia would be protected from fungicides, and could remain viable until ripening commenced.

It is tentatively suggested that the presence of an inhibitory substance in green apricot tissue may maintain the fungus in a latent condition. Evidence has been presented in this paper that a substance that inhibits the growth of a species of *Histoplasma* is present in green apricots. Satisfactory evidence has not yet been developed that this substance is inhibitory towards *S. fructicola,* but possibly it does not reach an effective concentration in the agar because of dilution or instability. It is also possible that it is more effective in the plant than *in vitro,* and may parallel the effect of streptomycin, which has been shown by Müller, Mackay, and Friend (1954) to confer resistance to *Phytophthora infestans* at concentrations in the plant that had no effect on the organism *in vitro.* The nature of the inhibitor has not been determined, but it is suggested that it may be either a substance of the antibiotic type or an organic acid. However, the latter seems unlikely on the ground of the evidence of instability provided by the absence of an inhibitory effect from macerated tissue. Substances with antibiotic activity towards plant pathogenic organisms have been found in a number of plants. Van Burgh (1952) suggested that the solanine content of green tomatoes accounts, in part at least, for the failure of the anthracnose organism to colonize green fruit after latent infection occurs. Inhibitors to *Bacterium stewarti* and *E. carotovora* in Iowa Inbred maize, to *E. carotovora* in Ohio 31 cucumber, to *X. campestris* in Wisconsin Jersey Queen cabbage and wild mustard, and to *Fusarium conglutinous* and *F. bulbigenum* var. *lycopersicae* in Pan American and Break o' Day tomatoes were found by Little and Grubough (1946). Irving *et al.* (1945) found an inhibitor to *F. bulbigenum* var. *lycopersicae,* which they named lycopersicin, in juice of Pan American tomatoes, and Johann and Dickson (1945) found that the inhibiting effect of juice of cornstalks to *Diplodia zeae* showed seasonal changes. It is suggested that an inhibitor of a similar nature is present in green apricots.

Latent infection of apricot fruit with *S. fructicola* explains the importance of early season fungicidal sprays for control of brown rot on fruit at harvest far more satisfactorily than the previous opinion that they exerted their effect indirectly by controlling blossom and twig infection and thus reduced the amount of inoculum present when the fruit ripened. It also explains why pre-harvest sprays have given disappointing results in control of brown rot in storage, and why brown rot can cause serious loss in a season when weather conditions at harvest are not favourable for infection. Further investigations to determine the period when latent infection is initiated, and to determine how to prevent its occurrence by use of fungicides, should result in greatly improved control.

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Mr. A. G. McIntyre, Division of Mathematical Statistics, C.S.I.R.O., kindly made a statistical examination of the results reported in Table 2.

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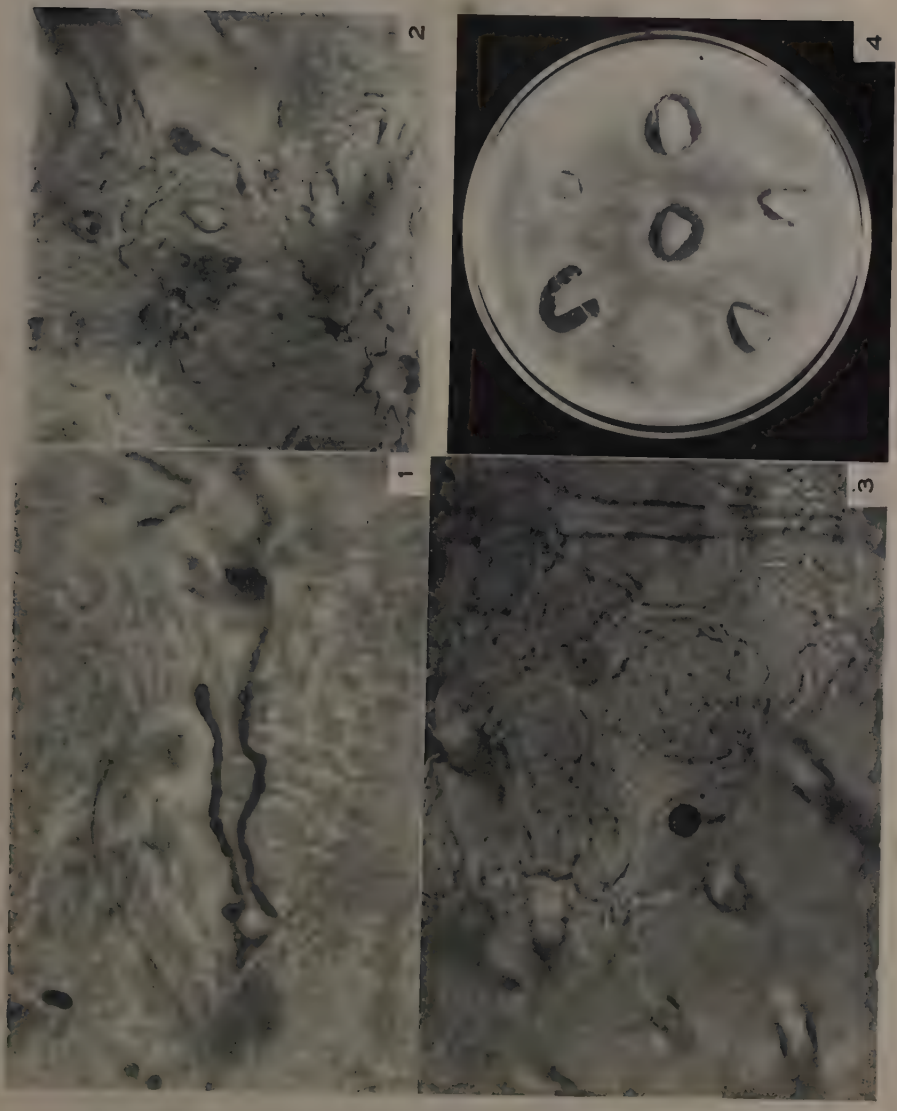
BROWN ROT OF APRICOTS. I



Explanation of Plate 1

Fig. 1.—Hyphae of *S. fructicola* growing on the epidermis of green apricot fruit. Stained with lactophenol–cotton blue. × 800.

Fig. 2.—Hypha of germinated conidium of *S. fructicola* growing towards a stoma of a green apricot fruit. × 500.

Fig. 3.—Conidium of *S. fructicola* lying within a stoma of a green apricot fruit. The conidium has germinated and penetrated a cell surrounding the stomatal cavity. × 500.

Fig. 4.—Zones of inhibition towards *Histoplasma* sp. around pieces of green apricot tissue.

INVESTIGATIONS ON BROWN ROT OF APRICOTS CAUSED BY *SCLEROTINIA FRUCTICOLA* (WINT.) REHM.

II. THE RELATIONSHIP OF THE POTASSIUM STATUS OF APRICOT TREES TO BROWN ROT SUSCEPTIBILITY

By G. C. WADE*

(*Manuscript received April* 23, 1956)

Summary

It is shown that under Tasmanian conditions there is a negative relation between the potassium status of apricot trees and their susceptibility to brown rot. From regression equations calculated, it is suggested that trees with a potassium content of about 4.6 per cent. in oven-dry leaves would be resistant to brown rot. Difficulty was experienced in increasing the potassium content of the trees by soil or spray applications of potassium fertilizer, but when this was achieved, some reduction in brown rot incidence occurred. Regular annual soil dressings of potassium chloride at the rate of 2 lb per tree was the most satisfactory treatment tested.

I. INTRODUCTION

In a previous paper (Wade 1952) data were presented which suggested that fruit from apricot trees of low potassium status were highly susceptible to brown rot. In the two experiments described in that paper a significant reduction in the percentage of brown rot was achieved by some soil or spray treatments with potassium chloride.

Investigations on this suggested relationship were continued in the seasons 1952-53 to 1954-55 inclusive, and the results are reported in the present paper, together with further information, not previously reported, on the 1951-52 experiments. A negative regression between the potassium content of the leaves and the percentage of brown rot is shown in all seven experiments conducted, and other information in support of the hypothesis will be presented.

II. DETERMINATION OF REGRESSIONS OF BROWN ROT ON POTASSIUM CONTENT

In the course of experiments, which will be described later, designed to test the effect of potassium applications on brown rot control, a record of the incidence of brown rot in the entire crop from each tree and an analysis of the potassium content of leaf samples taken in February were obtained.

With one exception, the experiments were conducted on the properties of Messrs. T. D. Calvert, Rokeby, and S. Haynes, Sandford. The soils

* Department of Agriculture, Hobart, Tas.

on those two properties are black clay loams and the chemical characteristics of the surface horizon of the soils are shown in Table 1. The other experiment was conducted on the property of Mr. R. Calvert, Sandford. The soil was a sandy loam.

In all experiments the trees were of the Moorpark variety, on myrobolan plum stocks. Leaf samples were taken in late February. Each sample consisted of approximately 200 leaves of intermediate age, taken from current season laterals from all positions on the tree. The figures for potassium are quoted as percentages on the dry weight of the leaves.

TABLE 1

CHEMICAL CHARACTERISTICS OF SOILS

Soil Characteristics	Property	
	S. Haynes	T. D. Calvert
pH	8.2	8.3
Available calcium	Very high	Very high
Exchangeable potassium (m-equiv.)	0.66	0.46
Total potassium (%) (Lawrence Smith)	0.77	0.72

In the 1951-52 and 1952-53 seasons the fruit ripened over a long period, so that three separate picks were necessary. As conditions during the periods varied in suitability for brown rot development, an estimated mean was calculated which represented the percentage of brown rot which would have developed if severity at the first and third picks had been the same as at the second. In the other seasons only two picks were necessary, and the brown rot was calculated from the totals of the two picks.

Regression equations were calculated by a method described by Snedecor (1940); the results are shown in Table 2, and a representative experiment illustrated graphically in Figure 1.

Thus in all the experiments there was a negative regression between brown rot incidence and potassium content of the leaves, and with one exception the regressions were statistically significant. Although the experiments were conducted in different seasons on different properties, there is reasonable agreement between the calculated potassium contents at which brown rot would not occur. The figure of 5.8 per cent. was obtained from results from a smaller number of trees, and the experiment was conducted on a different soil type.

The nitrogen contents of the leaves of trees in Mr. T. D. Calvert's orchard were determined in the 1951-52 season, but no relationship between nitrogen and brown rot susceptibility could be demonstrated.

TABLE 2

REGRESSIONS OF BROWN ROT ON POTASSIUM CONTENT OF LEAVES

Season	Orchard	No. of Trees	Range of K Content (x) (%)	Range of Brown Rot (E) (%)	Regression Equation	Significance of Equation (P)	Theor. K for No Brown Rot (%)
1951-52	R. Calvert	14	1.46-2.97	3.4-41.4	$E = 28.74 - 4.95x$	0.05	5.8
1951-52	T. D. Calvert	48	1.62-2.78	5.9-63.8	$E = 61.66 - 14.33x$	0.05	4.3
1952-53	T. D. Calvert	34	1.41-2.63	7.4-83.0	$E = 130.6 - 38.4x$	0.01	3.4
1952-53	S. Haynes	48	1.27-2.72	15.8-73.7	$E = 69.4 - 14.4x$	0.05	4.8
1953-54	T. D. Calvert	42	1.24-3.8	0.0-70.7	$E = 40.1 - 8.7x$	0.05	4.6
1953-54	S. Haynes	48	1.33-2.90	8.3-55.0	$E = 43.0 - 9.7x$	0.05	4.4
1954-55	S. Haynes	48	1.00-2.41	6.6-68.2	$E = 31.8 - 3.1x$	N.S.	—

A limited amount of information on the relation of the potassium content of fruit to brown rot incidence has also been obtained in the 1953-54 and 1954-55 seasons. Fifty fruit were sampled at random from

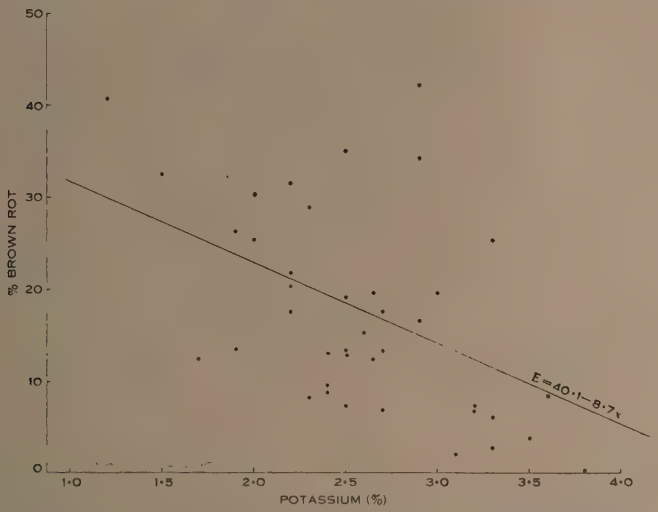


Fig. 1.—Brown rot percentage plotted against percentage of potassium in oven-dry leaves. Experiment conducted on Mr. T. D. Calvert's property, 1953-54.

each tree at harvest time, and cork-borer samples removed from each and oven-dried. The percentage of potassium on an oven-dry basis was then determined. Regression equations were determined and the results

TABLE 3

REGRESSIONS OF BROWN ROT ON POTASSIUM CONTENT OF FRUIT

Season	No. of Trees	Range of K Content (x) (%)	Range of Brown Rot (E) (%)	Regression Equation	Significance of Equation
1953-54	21	1.2-1.7	9.0-44.2	$E = 30.8 - 7.4x$	N.S.
1954-55	12	1.15-1.49	13.1-68.2	$E = 109.4 - 62.3x$	$P = 0.01$

are shown in Table 3. Both samples were taken on the property of Mr. S. Haynes, Sandford.

Although negative regressions were demonstrated in both seasons the regression was only significant in one season.

(a) *Potassium Content of Trees of Low Susceptibility*

Occasional trees which develop very little brown rot have been found in orchards which suffer heavy losses from brown rot. Analysis of the leaves of such trees have shown that they have a high potassium content. A tree with 2 per cent. brown rot on one property had 4.1 per cent. potassium in the leaves, and on another property two trees with less than 1 per cent. brown rot had 4.0 and 4.3 per cent. potassium respectively.

One orchard at New Norfolk has been irrigated for several years, and is now almost free from brown rot, although previously it was subject to heavy infection. Analysis of leaves from trees in that orchard showed a potassium content of 4.4 per cent.

III. Attempts to Control Brown Rot by Fertilizer Treatment

Experiments were conducted on a number of properties to determine the effect of addition of potassium fertilizers either as soil or spray applications on the incidence of brown rot, and on the potassium content of the leaves.

(a) *Experiment on the Property of Mr. T. D. Calvert, Rokeby*

The experiment was set up in 1951 and continued until the 1953-54 season, when it had to be abandoned because of loss of trees from *Libertiella* dieback. Seven replicates of seven treatments were arranged as a latin square, the same trees being used in each season to determine residual effects of previous treatments.

In the 1951-52 and 1952-53 seasons soil dressings were distributed evenly on the surface of the soil between trees, but in the 1953-54 season two treatments were included in which the dressing was distributed between approximately 20 holes, each 10 in. deep, around the periphery of the trees. When a single spray of 1 per cent. potassium chloride was used, it was applied in December. Four sprays were applied at monthly intervals commencing in September.

The results and treatments are shown in Table 4. The rate of application is shown on a per tree basis. Thiram was applied at petal fall and for three cover sprays commencing in late December.

In general, treatments which significantly increased the potassium content of the leaves usually significantly reduced the incidence of brown rot. However, no treatment raised the potassium level to a point approaching the theoretical level when brown rot would not occur, or to that of non-susceptible trees found in a number of orchards. Although the potassium chloride sprays reduced brown rot in 1951-52 they also caused necrotic spotting of the leaves, and would not therefore be satisfactory for general use by orchardists.

TABLE 4

EFFECT OF POTASSIUM APPLICATIONS ON BROWN ROT: ROKEBY EXPERIMENT

	A	B	C	D	E	F	G	Min. Sig. Diff.	
Treatment, 1951-52	Nil	2 lb KCl	4 lb KCl	2 lb $(NH_4)_2SO_4$	2 lb $(NH_4)_2SO_4$ + 2 lb KCl	1% KCl (1 spray)	1% KCl (4 sprays)	At 5% level:	At 1% level:
Brown rot (%)	40.46	28.76	24.83	39.50	32.96	18.73	25.73	13.16	17.81
Potassium (%)	1.98	2.18	2.22	2.01	2.23	2.38	2.47	0.22	0.29
Treatment, 1952-53	Nil	Nil	4 lb KCl + 1% KCl (1 spray)	2 lb KCl	2 lb KCl	Thiram	Thiram + 1% KCl (1 spray)		
Brown rot (%)	52.2	43.9	55.0	56.5	50.1	39.1	39.8	12.1	
Potassium (%)	1.81	2.13	2.25	1.93	2.22	—	—	N.S.	
Treatment, 1953-54	Nil	Nil	4 lb KCl (below surface)	2 lb KCl	2 lb KCl	2 lb KCl	4 lb KCl (below surface)		
Brown rot (%)	29.8	14.1	11.0	15.6	11.1	21.6	20.7	14.1	
Potassium (%)	1.7	2.3	2.7	2.7	2.9	2.3	2.7	0.26	0.36

(*b*) *Experiment on Property of Mr. S. Haynes, Sandford*

This experiment was commenced in the 1952-53 season. It was designed with six replicates of eight treatments. Because of the leaf injury caused by 1 per cent. potassium chloride sprays, 1 per cent. potassium sulphate was tested in 1952-53 and 1953-54. In 1954-55 a mulch of oaten hay at the rate of 30 lb per tree was used alone in one treatment, and in addition to a soil dressing of 2 lb potassium chloride per tree in another. Mulch treatments were found effective in controlling potassium deficiency of apples by Walsh and Clark (1950). The treatments and results are shown in Table 5.

None of the treatments was effective in raising the potassium status of the trees very greatly, and except for treatment E in 1953-54 and B in 1952-53 no significant reductions in brown rot incidence were achieved.

(*c*) *Other Trials*

In 1953-54 six trees in a different portion of Mr. S. Haynes's orchard from that used for the experiment just described were sprayed with 1 per cent. potassium sulphate 1 month before picking, and six untreated trees were left as control. No significant reduction in brown rot incidence (16.5 per cent. in untreated, compared with 17.6 per cent. in treated trees), or improvement in potassium content (1.7 and 1.8 per cent. respectively), was achieved.

In 1954-55 2 lb of potassium chloride per tree was distributed between 20 holes each 10 in. in depth, around the periphery of six trees on the property of Gill Bros., Cambridge. Six untreated trees were left as controls. The treatment resulted in a reduction, significant at the 5 per cent. level, from 7.2 per cent. brown rot to 4.0 per cent.

IV. Discussion

In seven experiments conducted over a 4-year period a negative regression between brown rot incidence and the potassium status of apricot trees was demonstrated in all cases. The regressions were statistically significant at either the 5 or the 1 per cent. level in six out of the seven experiments. The statistically significant regression equations were similar, and suggested that if the potassium content of apricots could be raised to about 4.6 per cent. in oven-dry leaves, brown rot should not occur. Although it is appreciated that other factors may modify the relationship, some support for this suggestion has been obtained from analysis of leaves from trees with very low brown rot incidence. Such trees have shown a potassium content close to the theoretical figure.

None of the trees in the properties where the work was conducted showed any of the usual symptoms of potassium deficiency, and their potassium content was above the level where deficiency would be expected.

TABLE 5

EFFECT OF POTASSIUM APPLICATIONS ON BROWN ROT: SANDFORD EXPERIMENTS

	A	B	C	D	E	F	G	H	Min. Sig. Diff.	
Treatment, 1952-53	Nil	2 lb KCl	2 lb KCl + KCl spray	4 lb KCl	4 lb KCl + KCl spray	1% KCl (1 spray)	1% K_2SO_4 (1 spray)	1% K_2SO_4 (4 sprays)	At 5% level:	At 1% level:
Brown rot (%)	50.3	29.8	42.1	41.6	41.3	41.1	50.6	43.3	16.8	
Potassium (%)	1.73	1.80	2.03	2.11	2.08	1.67	1.65	1.85	N.S.	
Treatment, 1953-54	Nil	Nil	2 lb KCl	Nil	2 lb KCl (below surface)	2 lb KCl	2 lb KCl (below surface)	K_2SO_4 (4 sprays)		
Brown rot (%)	27.3	24.8	17.1	22.9	15.1	25.1	20.1	25.7	11.1	
Potassium (%)	1.6	1.8	2.2	2.1	2.3	1.9	2.2	2.1	0.27	0.36
Treatment, 1954-55	Nil	2 lb KCl + mulch	2 lb KCl	Mulch	4 lb KCl (below surface)	Nil	2 lb KCl (below surface)	2 lb KCl (below surface)		
Brown rot (%)	33.2	28.7	21.5	39.4	29.7	31.7	28.1	20.6	N.S.	
Potassium (%)	1.7	1.8	2.2	2.1	2.3	1.9	2.2	2.1	0.27	0.37

Further, the exchangeable potassium of the soils was above the level of 0.4 per cent. m-equiv., which Leeper (1948) considers is the maximum at which potassium deficiency may be expected.

Difficulty was experienced in raising the potassium status of trees by application of potassium fertilizers either by soil dressings or sprays. In no case did the potassium content of the trees, after any of the treatments tested, approach the theoretical level at which brown rot should not occur. Nevertheless, if a treatment significantly raised the potassium content of the leaves, brown rot was usually reduced significantly. The most satisfactory treatment was annual soil dressings of potassium chloride at 2 or 4 lb per tree for at least two successive seasons. However, probably as a result of unfavourable weather conditions in some seasons, results were not always obtained by such treatment. (Mile (1936) found that worth-while results in the reduction of fusarium wilt of cotton by potassium fertilizer could not be obtained in dry seasons.) In some seasons spray applications of potassium chloride or potassium sulphate were effective. However, potassium chloride caused necrotic spotting of the leaves and would therefore not be suitable for general use, while potassium sulphate as a spray only gave significant results on one occasion.

Following the earlier report (Wade 1952) of a suspected relationship between potassium and brown rot, Hutton and Leigh (1955) tried soil and spray applications of potassium chloride against brown rot of peaches in New South Wales. They obtained no reduction in incidence. However, it is apparent from their quoted potassium analyses that the treatment did not raise the potassium content of the trees to a level where a marked effect could be expected. In Victoria (Fish 1955) numerous experiments have failed to show any relationship between the potassium content of stone fruit and their susceptibility to brown rot. It therefore appears that the relationship may not apply under all climatic conditions or in all soil types.

Potassium has been shown to affect the incidence of a number of plant diseases, e.g. fusarium wilt of cotton (Mile 1936), white root rot of onions due to *Sclerotium cepevorum* (Asthana 1945), maize wilt due to *Posudomonas stewarti* (Shear and Wingard 1944), barley mildew (Hopfergart 1953), bacterial wilt of tomato (Gallegly and Walker 1940), bacterial leaf spot of plums (Thomas, Mack, and Fagan 1947, 1948), *Aphanomyces* root rot of peas (Wade 1955), and *Phytophthora infestans* (Alten 1940).

Various suggested explanations of the effect of potassium on disease susceptibility have been offered. Shear and Wingard (1944) suggested that the increased susceptibility of potassium-deficient maize to bacterial wilt may be related to nitrate accumulation. Alten (1940) found a correlation between resistance of potatoes to *Phytophthora infestans*, as

affected by potassium fertilizers, and the content of non-protein and amino nitrogen. He found that the arginine content of tubers increased with the increase of potassium, and that arginine was toxic to the sporangia.

It appears from the results reported in this paper that the relatively low potassium status of apricots in Tasmania explains at least in part their high susceptibility to brown rot.

V. Acknowledgments

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DEFINITION OF POTATO VIRUS X STRAINS PRESENT IN NEW ZEALAND POTATO VARIETIES

By A. D. THOMSON*

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Summary

Thirteen different isolates of virus X were made from the main New Zealand potato varieties. Twelve of these proved to be constant strains and the reactions of a number of plant species to them have been studied. All strains gave precipitin reactions with an antiserum prepared against one of them. Inoculation of *Datura stramonium* L. plants with an avirulent strain prevented their infection with the other strains.

The relative severity of the strains on a number of test plants was similar. *Nicotiana glutinosa* L. was found to be satisfactory for the isolation and differentiation of most strains. *Gomphrena globosa* L. reacted with local lesions to all the strains. The symptom pictures of the strains have remained constant for 2 years except for one strain (X1), which showed a marked change in symptoms after 3 months in a deep-freeze unit.

One isolate, AST1, contained both mild and severe components. Its passage through *Cyphomandra betacea* Sendt. resulted in the selection of a severe type. The same isolate contained a component which systemically invaded *Chenopodium album* L. No other strain produced systemic symptoms on this plant.

I. INTRODUCTION

Strains of potato virus X were first recognized by Johnson (1925) and a number of subsequent studies have been made (e.g. Salaman 1938; Matthews 1949*a*, 1949*b*; Ladeburg, Larson, and Walker 1950).

Salaman (1938) classified his isolates into strains X^H, X^G, X^L, X^S, X^N, and X^D, depending on the reactions of certain potato varieties and indicator plants. A similar classification was made by Clinch (1944) who included virus B as a strain of virus X (X^B). However, it has been shown that many strains of virus X exist, many more in fact than Salaman recognized (Matthews 1949*a*; Ladeburg, Larson, and Walker 1950).

Naturally infected potato varieties are commonly infected with strain mixtures (Salaman 1938; Bald and White 1942; Matthews 1949*a*). Numerous inoculations of leaf tissue from important New Zealand potato varieties Dakota Red, Aucklander Short Top, and Arran Chief to *Nicotiana tabacum* L. (var. White Burley) have indicated that these varieties are infected with a mixture of predominantly mild, with some severe, strains. Plate 1, Figure 1, shows the reaction of *N. tabacum* to a typical Aucklander Short Top isolate.

* Crop Research Division, Department of Scientific and Industrial Research, Lincoln, New Zealand.

The strains studied in the present work were isolated from the three most widely grown potato varieties in New Zealand. One strain, however, has been isolated from the variety Craig's Bounty and one from a seedling introduced from the United States. No attempt was made to equate the strains isolated to those previously described, although most of Salaman's (1938) general groupings have been recognized.

II. Materials and Methods

(*a*) *Isolation*

Standard sap-inoculation techniques were used with fine-grained "Carborundum" powder to facilitate transmissions. All plants used were grown in steam-sterilized soil and maintained in insect-proof glass-houses. Water-inoculated controls were kept in the various experiments and these remained uninfected.

All potato samples were first inoculated to *Datura stramonium* L. to eliminate virus Y from virus X + Y complexes. From *Datura*, inoculations were made to *N. glutinosa* L. or *N. tabacum*. *N. glutinosa* was found satisfactory for the isolation and differentiation of ringspot isolates of virus X. Single lesions or portions of leaves of *N. glutinosa* were removed with a fine cork-borer, and the sap extracted, diluted with water, and inoculated to further plants. This procedure was repeated up to seven times. Table 1 indicates the source of the strains and the method of isolation.

All strains were maintained by serial transfer in indicator plants (mainly *N. glutinosa*) and in leaf material kept in a deep-freeze unit.

(*b*) *Cross-protection and Serological Studies*

All strains gave a precipitin reaction with an antiserum which was prepared against X4 by inoculating a rabbit with a series of intravenous injections of clarified virus preparation.

Avirulent strain AST4, when inoculated to *D. stramonium*, produced no visible symptoms, except occasionally a few necrotic flecks on the inoculated leaves. Avirulent strain infection in *D. stramonium* was shown by serological reactions and cross-protection tests.

A study was made of the cross-protection reactions of each strain against AST4. Young *D. stramonium* plants were inoculated with AST4, and 14 days later each strain was inoculated to 5 of these plants and 8 plants were inoculated with water; these latter remained symptomless. At the time of the second inoculation 3 uninoculated plants were inoculated with each strain; normal strain symptoms developed on these plants.

Strains 3XE-1, D1, X1, and CB gave a small number of ringspot lesions on the old inoculated leaves of the plants protected by AST4. However, no systemic symptoms developed. All other strains produced no response upon the protected plants.

TABLE 1

ORIGIN, ISOLATION, AND RELATIVE SEVERITY OF POTATO VIRUS X STRAINS

Strain*	Potato Variety from which Isolated	Origin	Symptoms on Potato Source	Indicator Plant Used for Isolation	Method of Isolation	Strain Type and Severity on *N. glutinosa*
AST1†	Aucklander Short Top	Dunedin	Faint mottle	*N. tabacum*	Single lesion dissection	Faint mottle
AST4	Aucklander Short Top	Lincoln	Faint mottle and leaf crinkle‡	*D. stramonium*	Whole leaf	Masked
D1	Dakota Red	Auckland	None	*N. glutinosa*	Single lesion dissection	Severe necrotic ringspot
D2	Dakota Red	Lincoln	None	*N. tabacum*	Whole leaf	Faint mottle
D3	Dakota Red	Lincoln	None	*N. glutinosa*	Single lesion dissection	Severe necrotic ringspot
AC1	Arran Chief	Pukekohe	Plant stunted, dark green	*N. tabacum*	Single lesion dissection	Ringspot
AC5	Arran Chief	Pukekohe	Slight mottle	*N. tabacum*	Single lesion dissection	Ringspot
X1	Unknown	P.D.D.§	Not recorded	*N. glutinosa*	Single lesion dissection	Necrotic, then change to ringspot
X2	Unknown	P.D.D.§	Not recorded	*N. glutinosa*	Single lesion dissection	Necrotic ringspot
X3	Unknown	P.D.D.§	Not recorded	*N. glutinosa*	Single lesion dissection	Ringspot
X4	Unknown	Lincoln	Not recorded	*N. glutinosa*	Single lesion dissection	Ringspot
CB	Craig's Bounty	Lincoln	Not recorded	*N. glutinosa*	Single lesion dissection	Ringspot
3XE-1	U.S.D.A. seedling 3XE-1	U.S.A.	Severe necrosis and mottle	*N. tabacum*	Whole leaf	Severe necrotic ringspot

* Initials refer to variety or seedling from which virus was isolated. Strains isolated from unknown potatoes are referred to as X1, X2, etc.

† This isolate, originally thought pure, was later shown to be a strain mixture.

‡ Symptoms due to virus X + Y complex.

§ Originated from isolates from Plant Diseases Division, D.S.I.R., Auckland.

III. Results

(a) *Reactions of a Selection of Plant Species to the Different Strains*

(i) *Nicotiana glutinosa.*—This plant was found useful for the differentiation of most of the strains. Strains which produced similar symptoms on this plant, namely, 3XE-1 and D3, X3, X4, and CB, were separated by the reactions of *Solanum nigrum* L., *S. dulcamara* L., and *S. nodiflorum* Jacq. (see Table 3) to them. Although symptoms tended to be more severe under winter conditions, strain differences were maintained.

Table 2

Reactions of *N. glutinosa* to potato virus X strains

Strain	Reaction on Inoculated Leaves	Systemic Reaction
AST4	No symptoms	No symptoms
AST1	Few faint chlorotic-yellow flecks and rings	Faint mottle and a few chlorotic flecks
3XE-1	Large irregular ringspots, tending to be necrotic in winter	Mottle, necrosis, and etched rings and ringspots
AC1	Large ringspots	Mottle and slight etching
AC5	Few ringspots	Mottle and irregular etch
D1	Rings and ringspots, typically more than 1 ring, rather necrotic	Bright mottle, etch, and some necrosis
D2	Occasionally a few faint ringspots	Faint mottle, few chlorotic flecks
D3	Rings and ringspots tending to be necrotic in winter	Similar to 3XE-1
X1	Ringspots	Mottle, rings, ringspots, and etched lines
X2	Necrotic ringspots	Mottle, etch
X3	Ringspots	Mottle, etch
X4	Ringspots	Mottle, etch
CB	Ringspots	Mottle, etch

Table 2 indicates the local and systemic symptoms on *N. glutinosa.*

Plate 1, Figure 2, shows the systemic reaction of *N. glutinosa* to a selection of strains.

However, during the course of these experiments a marked change took place in X1. This strain was originally isolated from a black necrotic lesion on *N. glutinosa* and produced characteristic local necrotic lesions and systemic necrosis on *N. glutinosa, N. tabacum,* and *D. stramonium.* Cross-protection and serological tests showed that this isolate was a strain of virus X. After a period of 3 months in the deep-freeze unit the symptom picture changed with the production of ringspot-strain

symptoms on the above three plants. No reversal to the original necrotic symptoms has taken place over an 18 month period and the isolate has been maintained as a ringspot strain.

The reactions of AST1 on *N. glutinosa* indicated that this isolate was probably a mixture of mild and severe strains, although predominantly mild.

(ii) *Datura stramonium.*—All strains isolated, except AST4, produced symptoms which varied from mild mottles to severe necroses. An attempt was made to compare the strains isolated with those described by Salaman (1938) using this plant. Each strain was inoculated to five plants, and although no accurate comparison can be made without Salaman's original isolates, it appeared possible to group the strains approximately thus: X^H type—AST4; X^L types—AST1, D2, AC1; X^S types—D1, AC5, X1, X2, X3, X4, CB; X^N types—D3, 3XE-1.

(iii) *Gomphrena globosa* L.—This plant reacted to all strains (Wilkinson and Blodgett 1948) with the production of local lesions with a chlorotic centre and a conspicuous red margin. No strain differences were observed on this plant.

(iv) *Solanum* spp.—Strains were inoculated to plants of each of the following *Solanum* species: *S. dulcamara, S. nigrum, S. laciniatum* Ait., and *S. nodiflorum.*

S. laciniatum is one of the two New Zealand native members of the genus (Baylis 1954). *S. nigrum* is a common weed in New Zealand potato fields. As far as is known there is no published work on the reaction of *S. laciniatum* to virus X although Smith (1931) found that both *S. nigrum* and *S. dulcamara* were susceptible.

The reactions of these plants to a selection of strains is shown in Table 3. The strains listed were those which produced similar symptoms on *N. glutinosa.* Avirulent strain AST4 failed to produce symptoms on these plants. Symptoms on *S. nodiflorum, S. dulcamara,* and *S. laciniatum* faded as the plants grew, and in most cases finally disappeared. The relative severity of all strains, as indicated by intensity of symptoms and effect on plant growth, was approximately the same as their symptom severity on *N. glutinosa.*

(v) *Nicotiana rustica* L.—Ladeburg, Larson, and Walker (1950) have used *N. rustica* to differentiate ringspot isolates of virus X. All strains gave rise to various ringspot patterns on inoculated leaves, except AST1 and 3XE-1. AST1 gave no symptoms on inoculated leaves while 3XE-1 gave necrotic spots. Systemic symptoms varied, much systemic necrosis occurring with D3 and 3XE-1. AST4 was not inoculated to this plant.

(vi) *Chenopodium album* L.—This plant is also a common weed in New Zealand potato fields. All strains (except AST4 which gave no symptoms) produced local chlorotic ringspot lesions (Plate 2, Fig. 1).

In all cases leaf-drop followed infection but no systemic symptoms developed and no virus was recovered from uninoculated leaves except

TABLE 3

REACTION OF *SOLANUM* SPECIES TO POTATO VIRUS X STRAINS

(L), reaction on inoculated leaves; (S), systemic reaction

Strain: 3XE-1 Species	D3	X3	X4	CB
S. nigrum	(L) A few ringspots	(L) Faint ringspots	(L) No reaction	(L) No reaction	(L) Large irregular ringspots with numerous rings
	(S) Severe etch, mottle, and necrotic flecks	(S) Severe etch	(S) Severe etch, few necrotic lesions	(S) Mottle, etch, some vein-banding and leaf crinkle	(S) Few ringspots and etch
S. dulcamara	(L) Ringspots with single necrotic ring, necrosis along veins	(L) Ringspots with single necrotic ring	(L) As for D3	(L) As for D3	(L) As for D3
	(S) Few chlorotic flecks	(S) Few systemic ringspots	(S) No symptoms	(S) No symptoms	(S) Necrotic-chlorotic flecks
S. laciniatum	(L) Few necrotic spots	(L) As for 3XE-1	(L) No reaction	(L) No reaction	(L) No reaction
	(S) Mottle, necrotic flecks, and lines	(S) As for 3XE-1	(S) As for 3XE-1	(S) As for 3XE-1	(S) As for 3XE-1
S. nodiflorum	(L) Necrotic-chlorotic spots and ringspots	(L) Small chlorotic ringspots	(L) Faint ringspots	(L) Numerous chlorotic ringspots	(L) Chlorotic rings
	(S) Chlorosis along veins	(S) Necrotic ringspots and chlorotic spots	(S) Few chlorotic areas	(S) No symptoms	(S) No symptoms

from plants inoculated with AST1. These plants developed systemic lesions similar to those on the inoculated leaves.

(vii) *Cyphomandra betacea* Sendt.—Plants inoculated with X1 and X2 failed to develop symptoms and no virus was recovered. CB, D2, and AST4 were carried without the production of symptoms, and X3, X4, D1, D3, AST1, AC1, AC5, and 3XE-1 produced degrees of mottle symptoms.

(*b*) *The Separation of Virus X Strains from a Mixture*

Matthews (1949*c*) was able to separate severe virus X strains from a mixture by passage through *C. betacea*. Hutton (1948) made use of potato seedlings to separate mild virus X strains.

All *C. betacea* plants inoculated with the virus X strains in the present host-range studies were sampled and each was inoculated to two *N. glutinosa* plants. The original strain material was also inoculated to two *N. glutinosa* plants. No increase in severity or change in symptoms produced by the strains took place except in the case of AST1. Two young *C. betacea* plants were again inoculated with AST1. Seven weeks later young leaves were sampled and sap-inoculated to *N. glutinosa* with controls as before. Again there was a marked increase in the severity of AST1 after passage through *C. betacea* (Plate 2, Fig. 2).

The reaction of AST1 on *C. album* indicated that the isolate contained a component which systemically invaded this plant. In a further experiment 10 young *C. album* plants were each inoculated on three leaves with unselected AST1. AST1 ex *C. betacea* was also inoculated to three leaves on each of five plants. Numerous lesions developed on the inoculated leaves of all plants, and numerous systemic lesions developed on plants inoculated with unselected AST1. AST1 ex *C. betacea* showed only two leaves on one plant with systemic symptoms. This indicates that the component of AST1 systemic in *C. album* was not completely eliminated after passage through *C. betacea*.

The lesion-dissection technique was used to isolate virus from the systemic lesions induced by AST1 on *C. album*. Inoculum thus removed produced only faint mottle to vein-banding symptoms on *N. glutinosa*. No virus was recovered from the symptomless leaves. The recovered virus showed no indication of any severe strain remaining; however, further passage through *C. betacea* resulted again in the selection of the severe component.

The above experiments were carried out with young *C. album* plants and it was observed that older plants at the flowering stage gave only a few local lesions and resulted in no systemic spread with AST1.

IV. Discussion

It is apparent that, as in other countries, a wide range of potato virus X strains occur in New Zealand. There are a number of reports of avirulent strains similar to AST4 (Salaman 1938; Hutton 1948; Timian, Hooker, and Petersen 1955). Hutton and Peak (1951) were able to

identify differences between their avirulent strains by the reaction of *G. globosa* and *Capsicum frutescens* L. to them. In the present work three isolates similar to AST4 were found in the variety Aucklander Short Top, but no attempt was made to identify possible differences between these isolates. The occurrence of avirulent AST4 type strains tends to complicate testing work. However, it has been shown that this strain, as well as all others, produces definite localized symptoms on *G. globosa*.

All strains studied except X1 have shown no marked changes in symptom picture over the period during which they have been observed. Changes in the form of mutation or selection from a strain mixture would be difficult to observe with the severe strains. No changes in the avirulent strain (AST4) have been observed, although Hutton and Peak (1951) indicated that virulent mutants arose from time to time in their avirulent strains. There are a number of reports of high temperatures causing changes in viruses (Salaman 1937, 1938; Johnson 1947). Low temperatures have not been noted as causing changes in plant viruses. It is difficult to explain the apparent complete change in symptom picture of X1 but it may have been due to selection from a mixture of necrotic and ringspot strains.

The reactions of *C. album* and *C. betacea* to AST1 are of interest. It may be possible after a number of passages through these plants to separate completely the mild and severe components of AST1. Salaman (1938) presented some evidence that a change in virulence of his severe X^S strain to the milder X^G took place in horse-beans, red beet, and sugar beet. He recovered the milder virus from the local lesions on the inoculated leaves. With *C. album* a more complete separation would appear to be likely, since the virus moves away from the site of inoculation and forms systemic local lesions.

Chenopodium and *Cyphomandra* appear to be suitable plants for identifying certain mild and severe virus X strains in mixtures. In a study of virus cross-protection and possible recombination (Best 1954) such plants would be useful. It will be necessary, however, to study the physical and chemical characteristics of the strains to identify further suitable markers.

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POTATO VIRUS X STRAINS

POTATO VIRUS X STRAINS

1

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Explanation of Plates 1 and 2

Plate 1

Fig. 1.—The reaction of *N. tabacum* to a typical Aucklander Short Top isolate. Chlorotic and necrotic lesions indicate some severe virus X. Left, healthy control.

Fig. 2.—The systemic reaction of *N. glutinosa* to virus X strains. Top row, left to right: water-inoculated control, AST4, D2, AC1. Bottom row, left to right: CB, AC5, X4, 3XE-1, D1.

Plate 2

Fig. 1.—The local and systemic reactions of *C. album* to AST1. Left, healthy control. Centre, systemic lesions on young leaf. Right, local lesions on inoculated leaf.

Fig. 2.—Left, *Nicotiana glutinosa* inoculated with AST1 from *Cyphomandra betacea*. Centre, with original AST1 strain mixture. Right, with AST1 from *Chenopodium album*.

PARNA IN THE RIVERINE PLAIN OF SOUTH-EASTERN AUSTRALIA AND THE SOILS THEREON

By B. E. Butler* and J. T. Hutton†

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Summary

This study of Widgelli parna, aeolian material derived from soils during a previous arid period, shows that there is a relationship between particle size and the distance leeward from the dune zones. Within 1 or 2 miles of the dunes the modal size of the non-clay fraction is 150 to 200 μ, and this value changes to 40 μ at a distance of 150 miles leeward. The clay content varies inversely, being about 35 per cent. near the dunes and 70 per cent. 150 miles leeward. The non-clay fraction is highly graded. Within the dune zones, and particularly in the dune itself, the parna is mixed with coarser sand. The size and clay-content relationships for the parna sheet also hold in the dune zones with the modal size at the crest about 400 μ.

The landscapes and soils to be related to the parna include the riverine plains, the adjoining hills, and the dune landscape of the Mallee. Highly differentiated soils occur on the parna layer, and these include brown solonized soils in the dune zones, red-brown earths on the hills, and red-brown earths and grey and brown soils of heavy texture on the riverine plains. Soil differentiation is largely caused by drainage differences.

I. Introduction

Parna has been introduced (Butler 1956) as an aeolian clay the origin of which is to be related to arid climatic phases in the Quaternary. The material is distributed and deposited by wind and is comparable with loess, but it arises from the erosion of soils by wind. This paper follows on the previous paper and is a description of samples, and an analysis of the variation in particle size distribution. There is also a description of the soils and the landscapes from which the samples were taken and some mention of the underlying layers. The relationship between sand dune and parna sheet is examined. This study is confined to one parna sheet—the Widgelli parna (Butler 1956).

II. Sampling

The first series of samples were collected along an east-west traverse from Birchip in Victoria to Walbundry (near Corowa) in New South Wales. This sampling was made in the anticipation that the parna would show particle size variations with distance from the source similar to those found by Smith (1942) for loess. The locations of the samples are shown in Figure 1, which also shows the approximate distribution of the Widgelli

* Division of Soils, C.S.I.R.O., Canberra.

† Division of Soils, C.S.I.R.O., Adelaide.

parna (as it is known at present) in this part of Australia. Subsequent study has shown that the particle size properties of the parna were not related simply to distance eastward of a common datum, but to distance eastward of sand dunes. It was necessary, therefore, to go back into the

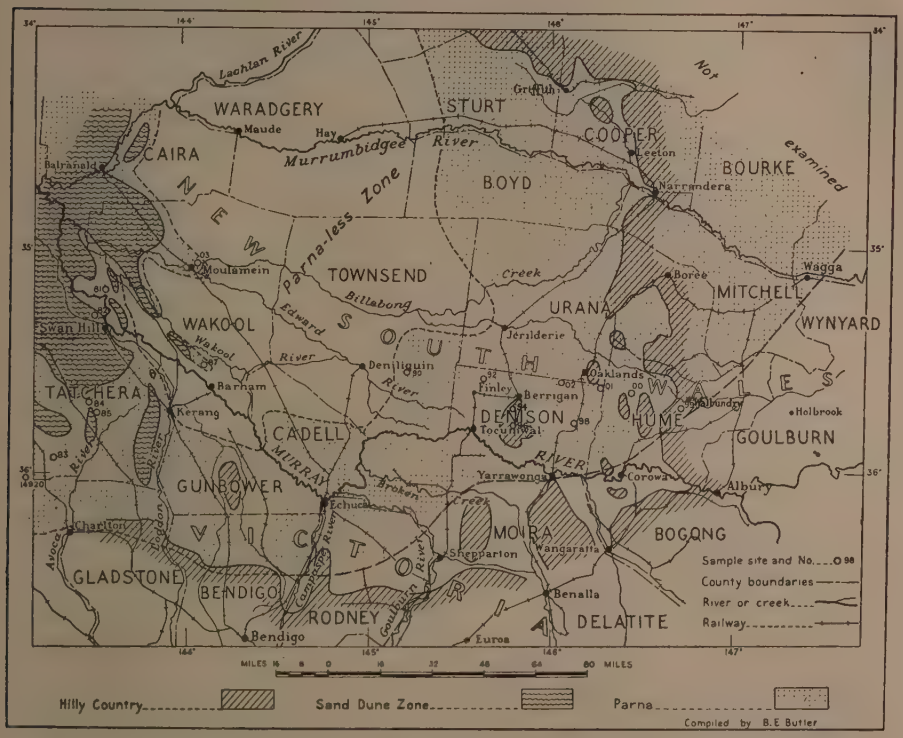

Fig. 1.—Map showing approximate distribution of Widgelli parna in the Riverine Plain of south-eastern Australia.

field and check these distances for each sample, and Figure 1 includes a delineation of the sand dune zones in proximity to the sampling sites. Table 1 is a summary of data for each parna sample from this sequence.

III. Thickness of the Parna Sheet

There is no regular variation in thickness of the parna sheet as found by Smith (1942) for loess. In the western part of the region the parna lies to a thickness of 3–10 ft in association with the sand dunes. Where the sand dunes become widely spaced, as to the east of Swan Hill, the parna sheet extends only for a few miles to the east of each dune. There is virtually no parna then for a further distance eastward of some 70 miles. This area is called the "parnaless" zone and was referred to earlier

(Butler 1956). Eastward of the parnaless zone the thickness of the sheet, as recorded in Table 1, is usually between 3 and 5 ft but shows no definite trends.

IV. The Nature of the Underlying Layer

All of the samples except sample 19001 were collected from the parna where it occurs as a surface exposure. The Widgelli parna is overlain by a riverine layer over considerable areas. Sample 19001 from County Denison is from such a site. This arrangement of depositional layers also occurs extensively in the Counties of Gunbower, Rodney, and Moira.

The layers underlying the Widgelli parna are commonly riverine sediments as is to be expected from the nature of the region (Butler 1950). These sediments may be grouped broadly into two:

(*a*) Those which are quite unweathered, loose, and crumbly.

(*b*) Those which are highly weathered, dense, and often superplastic (Butler 1955).

The field criterion of weathering is the absence of mica in the upper 3-4 ft of the layer. It is proposed to elaborate further on these riverine layers in another publication: it is sufficient to remark here that, as will be shown below, the nature of the underlying riverine layer—whether coarse or fine in texture, weathered or unweathered—has no effect on the particle size grading of the overlying parna sheet.

Samples 18994 and 18996 overlie the granite of the hills in County Denison. The substrate of one of these is a loose, micaceous material; the other is highly weathered and leached and consists of white "pipe clay". Both substrates contain coarse quartz grit from the coarse crystallinity of the granite but this fraction is absent from the overlying parna. A similar array of parna sheet and substrate has been observed on the granite hills in County Gunbower. Parna rests on quartzites and quartzitic conglomerates on the hills near Griffith and Urana and on the shale and chert of the hills to the south-east of Rochester. It seems that the hills east of Shepparton have not a cover of parna.

Samples 18984, 18985, and 19420 overlie earlier parna sheets, but these are not defined at the present time. This arrangement of layers is common in the Counties of Tatchera and in the northern part of Gladstone and Kara Kara and also in the hill sections of County Sturt. The lower parna sheets are frequently unrecognizable as soil surfaces except that they consist of a horizon depleted of lime overlying a lime-rich layer. It is possible that the underlying surface was eroded before the next parna was deposited on it. A profile through these layers shows a series of lime layers separated by horizons low in lime; the lime appears more definitely concretionary in each lower layer and is increasingly removed from the intervening fine earth. The fine earth is usually a red clay and each lower

layer is denser than the one above. The number of these parna sheets below the Widgelli parna may be as many as four, but usually only one or two can be recognized. Hills (1939) mentions two superposed soils with their lime horizons. The final substrate below the parnas in the regions just mentioned is often one containing concretionary or vesicular sesquioxide segregations, which may even be in the form of a boulder bed. This rests on a red and white mottled, dense, sandy clay. This appears to be a laterite profile and in the regions south-west of Swan Hill it appears to be formed on a fine sandstone which is possibly Pliocene in age. A good section through these layers can be seen in a railway cutting a mile to the south of Boort.

Samples 18999 and 19000 are taken from the eastern extremity of the parna sheet where it is found only in the valleys; it appears to have been eroded from the hills in this vicinity though it is to be found on hills further north at Lockhart.

All of the samples used in this study came from sheet parna and none from *dune parna* (Butler 1956).

V. The Soil Profiles on the Widgelli Parna

The soils on the Widgelli parna are well differentiated, both within the profile and between profiles on different parts of the landscape. Soil profile differences can be related to differences in drainage condition of the site, and to differences in the proportion of saltation sand in the parna. Details of the soil conditions of parna are discussed in current soil surveys which will be published in due course; it is intended here only to give the main trends in the range of soil conditions which occur.

(*a*) *Profiles on Pure Parna*

The term "pure parna" is defined as parna sheets removed more than 1 or 2 miles from the saltation zones, and not containing an obvious component of saltation sand; the characteristic sequence of soils is shown in Figure 2. The parna sheet landscape has no characteristic form, as the parna sheet blankets and assumes the form of the underlying landscape. The underlying landscape is a riverine plain with scattered hills, and has already been described (Butler 1950). The chief variations in the characteristic soil sequence are:

(1) The thickness of the textural A horizon, which varies from 9 in. to zero.
(2) The depth to lime, which varies from 36 in. to zero.
(3) The nature of the clay in the lower part of the profile: this may be highly subplastic or fully plastic (Butler 1955).
(4) The consistence of the surface layer, which varies from moderately dense to loose and self-mulching.
(5) The colour of the A and B horizons, which varies from red-brown to dark grey.

In general terms the profiles may be described as follows:

Profile A has an A horizon of brown (5YR5/4)* loam 6-9 in. thick, of weak ½ in. subangular blocky structure, slightly paler and with a platy structure in the lower 2 in. This changes suddenly to the B horizon, a strong red-brown (2.5YR4/4) heavy clay, of moderate grade ½ in. angular blocky structure. This material changes gradually at 18 in. to yellow-brown (5YR6/6) clay with a yellow shear. This clay is subplastic and the subplasticity usually becomes more marked with depth. Light amounts of soft lime and carbonate concretions occur from about 36 in. downward. An example of this profile is the Merungle loam (Taylor and Hooper 1938).

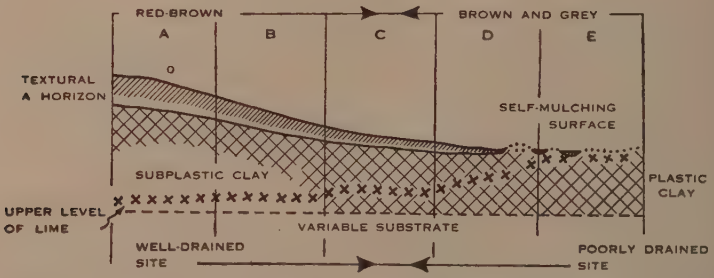

Fig. 2.—Diagrammatic scheme of the characteristic sequence of soils on the "pure" Widgelli parna.

Profile B is similar to profile A in colour and arrangement of horizons; however, the A horizon is only about 6 in. thick, and the subplastic property develops at about 30 in. and not to the high degree that it does in profile A. Lime comes in at about 30 in. Examples of this profile are Moira loam, friable subsoil phase (Butler *et al.* 1942; Smith 1945), and Thulabin loam (Churchward and Flint 1956). The description of soil A and soil B in the published record is rather variable owing to the various authors' methods of describing these subplastic horizons.

Profile C is again similar to profile B except that there is no development of the subplastic property. The A horizon is 4-7 in. thick, rather denser than in soils A and B and with a more consistent development of bleached A_2 horizon. There is a sharp transition to the B horizon, which is a strong red-brown heavy clay of moderate grade 1 in. angular blocky structure, very hard when dry and plastic when wet; and this continues to depth, becoming grey-brown (2.5Y5/4). Light amounts of lime appear at about 24 in. Profile C is a very widespread type and is represented by many profiles (though probably not all) of Moira loam (Butler *et al.* 1942), Birganbigal loam (Smith 1945; Churchward and Flint 1956), and Womboota loam (Johnston 1953).

* Munsell colour notation.

Profile D is similar to profile C in general respects, but is rather denser with a more plastic clay in the B horizon, the colour may be a duller brown, the thickness of the A horizon varies from 1 to 4 in., and the lime comes in at 12-18 in. Examples of this soil are Wilbriggie clay loam and Coree clay (Churchward and Flint 1956).

Profile E often occurs as a microcomplex with profile D, the two profiles being associated with microrelief; however, areas consisting entirely of profile E, both with and without microrelief, are encountered. Profile E may be dark grey-brown (10YR4/3) or dark grey (2.5Y4/2). There is usually little or no textural differentiation in the profile which, however, shows structural differentiation. The upper part is a strong grade of $\frac{1}{4}$ to $\frac{1}{8}$ in. subangular blocky structure which is loose and easily crumbled when dry but is soft and labile when wet. The structure deteriorates below the surface layer and by 12 in. it is a strong grade of 12 in. angular blocky structure, extremely hard when dry, firm and plastic when wet. Low amounts of carbonates occur from the surface or from within 9 in. of the surface. The colour value increases with depth, becoming grey-brown (10YR5/3) or grey (5Y5/2) by 36 in. Examples of profile E are Tragowel clay, self-mulching phase (Baldwin, Burvill, and Freedman 1939) and Wunnamurra and Yooroobla clays (Churchward and Flint 1956).

In all the preceding soils with a textural A horizon the pH of the A horizon is about 6.0, and builds up through 7.0 to about 8.6 at 6 or 12 in. inside the B horizon, and may rise to 9 with depth. In profile E the pH is 8.0 to 8.4 at the surface and rises quickly to 9 with depth.

It will be noted (Table 1) that all of the profiles in the characteristic sequence are represented in this sampling. The profiles described above refer to the soil condition in the parna sheet and this is most often 3-5 ft thick. In some cases the parna may not be thick enough to contain the whole profile, and some part of it (e.g. lime) may be missing from the parna sheet and leached into the underlying layer. The underlying layer may influence the nature of the soil profile in the parna by its effect on the drainage. Profiles A and B often occur on the sloping country associated with the hills, but equally well they occur overlying the levee formations of the Riverine Plain. In these latter cases the site may be only a foot or two above the general level of the plain, and yet the underlying coarse sediments have improved the drainage so that a soil comparable with that on the elevated, sloping land of the hills has developed. It may be presumed that the feature which these two sites have in common, viz. free drainage, is the causal factor of the soil condition. It has been noted, conversely, that profiles D and E may occur on flat sections of the hills as well as on the Riverine Plain overlying the dense, level-lying clays.

(*b*) *Profiles on Sandy Parna*

Parna is related to most of the sand dune country within the region. Within and near the sand dune landscape the parna is mixed with coarser sand in varying proportions. The proportion of sand varies in an orderly manner, being highest on the crest of the dune, decreasing down the slope of the dune, and being least in the interdune corridor. This variation may occur at any proportion of sand to parna, from perhaps 95 per cent. sand to 30 per cent. sand at the crest of the dune. The sandy parna landscape is characteristic in form, consisting of dunes tailing off into sheets. The development of the sheet is often interrupted by the repetition of the dune system.

The zone of sand dunes with parna is typical of the "Mallee country". In the region considered in this paper the Mallee zone is described and defined by Hills (1939). The main zones of sand dunes have an approximate north-south trend and these are indicated in Figure 1. These zones mainly correspond to the structural ridges mentioned by Hills; however, some of the ridges are not covered by sand (as, for example, the "Cannie Ridge" near Lalbert). Between the main sandy zones "pure" parna lies, and in the broad troughs it is underlain, and in part overlain, by riverine materials.

Within the sand dune zones the dunes have an east-west orientation and a characteristic sequence of soils is indicated for the transverse (north-south) traverse. The characteristic sequence of soils cannot be so confidently defined as that for the pure parna. In broad terms it is suggested in Figure 3. The main variables across the characteristic sequence of soils are in terms of:

(1) Variation in proportion of sand: the greatest amount occurring on the crest and the least in the hollow.

(2) The content of clay and carbonates, which vary together in the opposite manner from the sand component.

(3) The colour, which varies from red-brown to grey.

The most consistent difference between the profiles on pure parna from those on sandy parna is that in the latter the texture changes between A and B horizons are gradual. The higher the proportion of sand the more this tendency is developed. It is not proposed to describe profiles G, H, I, J, and K for this characteristic sequence, but examples may be quoted from Taylor *et al.* (1933) in which profile G would be represented by Tyntynder series, profile H by Tatchera series, profile I by Vinifera series, and profile J by Nyah series. It will be noted that this sequence of soils has no grey member. A comparable sequence of soils may be quoted from Taylor and Penman (1930) in which Type 1 corresponds with profile G, Type 8 with profile I, and Types 9 and 9A with profile K. In this sequence the grey member is present.

To examine the relationship between sand dune and parna sheet a series of samples was taken along a transect. This was chosen on the easternmost dune in a zone of sand dunes so that the transect could extend eastward (leeward) well into pure parna country. Such a site was found in the sand dune occurring in portion 39 of the Parish of Nullawil in the County of Tatchera, and samples were collected at the crest of the dune, and at $\frac{1}{8}$ mile, $\frac{1}{4}$ mile, $\frac{1}{2}$ mile, 1 mile, 2, 4, 8, and 16 miles distant. All of the samples were taken from the one depositional sheet and this overlay a second parna sheet at a depth varying between 2 and 4 ft. In the distant extremity the substrate was a riverine clay. The data for this series of samples are set out in Table 2.

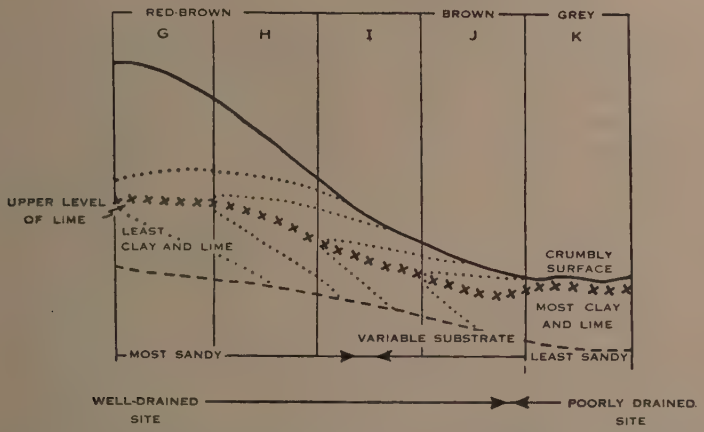

Fig. 3.—Diagrammatic scheme of the characteristic sequence of soils on the sandy parna in dune landscape. The dotted lines suggest isopleths of proportion of clay and lime.

VI. Laboratory Examination of Samples

Particle size distribution studies were made on the samples of parna collected in the original traverse and on the subsequent shorter traverse in the sandy parna zone. After removing particles greater than 2 mm all soils were treated with acid to remove any carbonate and then dispersed with sodium hydroxide and "Calgon" as described by Hutton (1955). To ensure adequate wetting and dispersion the normal overnight shaking in an end-over-end machine was replaced by shaking 2 hr every 8 hr for 3 days. The concentration of particles less than 2, 5, 10, 20, and 50 μ was determined by a plummet balance after appropriate settling periods (Hutton 1955). Sieves were used to separate the coarser material at 0.11, 0.15, 0.19, 0.40, and 0.80 mm. The presentation of the results of the particle size distribution studies has been discussed by many workers (Krumbein and Pettijohn 1938; Bagnold 1941; Doeglas 1946) without

TABLE 1

DESCRIPTION OF SAMPLES FROM WIDGELLI PARNA

Sample No.	Location: State, County, Parish, Portion	Distance E. from Sandhills (miles)	Thickness of Parna Layer	Description of Underlying Layer	Soil Profile Type	Depth of Sample (in.)	Colour (Munsell notation)	Clay (%)	Analysis of Non-Clay Fraction	
									Mode (μ)	Log Sorting Coefficient
18985	Vic., Tatchera, Talgitcha, 5	1	4 ft	Stump of older parna layer	C	24-30	7.5YR5/5	29	140	0.33
18982	Vic., Tatchera, Tyntynder West, 37	2	7 ft	Weathered, dense riverine clay	E	48-54	10YR6/4	40	250	0.26
18984	Vic., Tatchera, Gnarwee, 39	3	6 ft	Stump of older parna layer	C	60-66	5YR5/5	52	400	0.39
18987	N.S.W., Wakool, Danberra, 24	4	3 ft	Weathered, dense riverine clay	E	21-24	1.5Y6/3	42	200	0.41
18981	N.S.W., Wakool, Speewa, 2	10	4 ft	Weathered, dense riverine clay	E	36-42	2.5Y7/4	51	180	0.38
19003	N.S.W., Wakool, Moulamein, town	16	10 ft	Weathered, dense riverine clay	E	108-112	2.5Y7/2	53	110	0.37
19420	Vic., Tatchera, Kanyrie, 15	20	27 in.	Older parna layer	B	21-24	5YR5/6	55	190	0.33
18983	Vic., Tatchera, Cooroopajerrup, 2	20	8 ft	Weathered, dense riverine clay	B	84-90	10YR6/5	59	95	0.36
18990	N.S.W., Townsend, Thurgoon, 32	84	3 ft	Unweathered riverine sandy clay	D	24-30	2.5Y6/1	66	70	0.50
18992	N.S.W., Denison, Sargood, 13	110	3 ft	Unweathered riverine sandy loam	D	24-27	10YR5/3	64	60	0.51

TABLE 1 (*Continued*)

Sample No.	Location: State, County, Parish, Portion	Distance E. from Sandhills (miles)	Thickness of Parna Layer	Description of Underlying Layer	Soil Profile Type	Depth of Sample (in.)	Colour (Munsell notation)	Clay (%)	Analysis of Non-Clay Fraction	
									Mode (μ)	Log Sorting Coefficient
18994	N.S.W., Denison, Gereldery, 53	120	3 ft	Weathered granite	B	27-33	5YR5/6	46	100	0.58
18996	N.S.W., Denison, Kilnyana, 14	125	3 ft	"Pipe clay" granite	A	30-33	7.5YR5/6	54	42	0.34
18998	N.S.W., Denison, Bull Plain, 75	130	5 ft	Weathered, dense riverine clay	D	48-54	10YR6/5	66	42	0.35
19002	N.S.W., Denison, Narrow Plain, 27	130	3 ft	Unweathered riverine sandy clay	D	24-30	2.5Y5/3	69	60	0.44
19001	N.S.W., Denison, Coreen West, 26	140	12-30 in.	Unweathered riverine sandy clay		24-30	10YR5/3	64	75	0.30
19000	N.S.W., Hume, Osborne, 68	150	39 in.	Unweathered riverine sandy clay	A	30-36	7.5YR6/5	60	50	0.34
18999	N.S.W., Hume, Hindmarsh, 102	170	30 in.	Unweathered riverine sandy clay	C	24-30	7.5YR6/5	57	42	0.34

any agreement as to whether arithmetic, logarithmic, or probability scales should be used. The simplest and most satisfactory form of graphical presentation for the results available in this study was to plot on semilogarithmic paper the accumulated values of weight percentages less than a given size against the size of the fraction. Figure 4 shows the shape of some of the curves obtained.

The curves represent the particle size analysis of the material left after the acid treatment of the soils. The original parna contained some carbonate material but as there has been some redistribution of this, all particle size distribution figures quoted are on a carbonate-free basis.

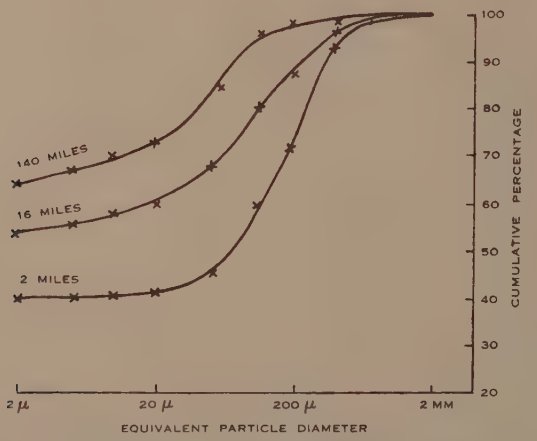

Fig. 4.—Summation curves showing particle size distribution for selected samples.

These summation curves have been used to derive size distribution curves and measures to indicate the average size and the degree of sorting of the particles greater than 2 μ. For this paper the average size has been defined by the mode, i.e. the diameter corresponding to the peak in the particle size distribution curve, and the degree of sorting by the logarithm of the geometric quartile deviation (log sorting coefficient) (Krumbein and Pettijohn 1938).

Table 1 gives descriptions of the samples collected from soils on the Widgelli parna together with the results for clay content, mode, and degree of sorting of the non-clay fractions. The relationships between distance eastwards from the dune zones and (*a*) clay content, and (*b*) modal size of the non-clay fraction of the samples, are shown in Figure 5. It will be seen that the modal size varies inversely with the distance from the dunes, being about 200 μ in the near vicinity and decreasing to 40 μ at distances of over 100 miles to the leeward. The clay content shows the opposite trend, being 30-40 per cent. close to the dunes and 60-70 per

cent. at 100 miles' distance. It is interesting that the trend in clay content is apparent in spite of the development of considerable texture differentiation in some of the profiles.

Table 2 and Figure 6 give the data for the samples associated with sandy parna and collected from the Parish of Nullawil.

The degree of sorting of the particles in the samples can be compared with figures given by Trask (1932) and quoted by Krumbein and Pettijohn (1938). Trask states that well-sorted material has a value for the logarithm of the geometric quartile deviation of less than 0.39 and poorly

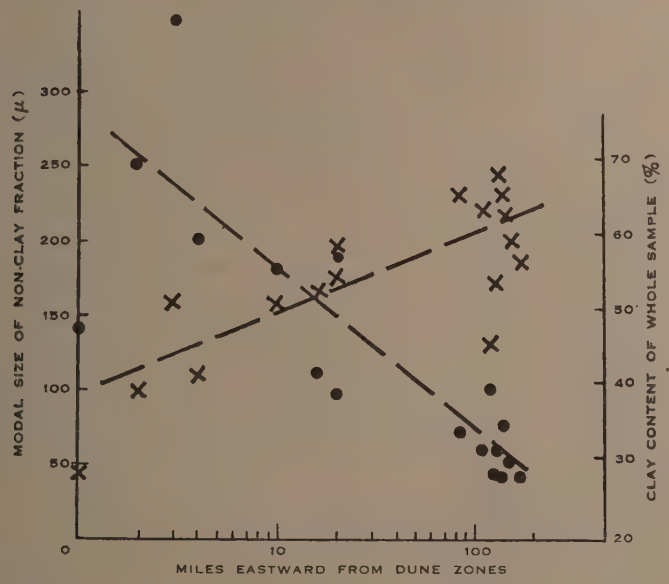

Fig. 5.—Particle size study of the Widgelli parna: size of non-clay fraction and percentage of clay (whole sample) plotted against distance eastward of dune zone. ● Modal size; × clay content.

sorted material a value greater than 0.65. It will be seen that the non-clay fraction of most of the parna samples can be classed as well sorted and none as unsorted. There is, however, no systematic variation in the degree of sorting with distance from the dune zone. The results when plotted on semilogarithmic paper indicate that there is usually positive skewness in the grading of samples taken near the sand dune zone, while the distribution of particles in samples taken at a great distance from the dunes appears to approach a symmetrical, normal curve. This trend, however, is not conclusively established on the present data.

TABLE 2

DESCRIPTION OF SAMPLES FROM SAND DUNE ZONE

Sample No.	Location: State, County, Parish, Portion	Distance E. from Sandhill (miles)	Thickness of Widgelli Parna Layer (in.)	Description of Underlying Layer	Soil Profile Type	Depth of Sample (in.)	Clay (%)	Analysis of Non-Clay Fraction	
								Mode (μ)	Log Sorting Coefficient
19421	Vic., Tatchera, Nullawil, 39	Zero on crest	36	Older parna stump	H	18-24	44	410	0.36
19422	Vic., Tatchera, Nullawil, 39	$\frac{1}{8}$	36	Older parna stump	I	21-27	43	370	0.25
19423	Vic., Tatchera, Nullawil, 39	$\frac{1}{4}$	24	Older parna stump	I	18-21	43	350	0.27
19424	Vic., Tatchera, Nullawil, 40	$\frac{1}{2}$	24	Older parna stump	I	18-21	34	300	0.25
19425	Vic., Tatchera, Nullawil, 40	1	33	Older parna stump	I	24-30	61	300	0.32
19426	Vic., Tatchera, Nullawil, 41	2	30	Older parna stump	J	21-27	52	330	0.29
19427	Vic., Tatchera, Nullawil, 43	4	24	Older parna stump	J	18-21	52	230	0.28
19428	Vic., Tatchera, Kalpienung, 18	8	24	Weathered riverine sandy clay	J	18-21	63	100	0.42
19429	Vic., Tatchera, Towaninny, 12	16	54	Weathered riverine heavy clay	K	24-30	69	90	0.38

The approach of Doeglas (1946) to the consideration of particle size distribution data is rather different from the generally accepted views, and he uses an arithmetic scale for the diameter of the particles and a probability scale for the summation percentages. Plots of the results obtained in this work give curves of Doeglas's "T type", which are claimed to be characteristic of loess, flood plain, and estuarine deposits. There is also a suggestion of a variable development of a coarse "tail"—a minor proportion of coarse material added to the main population—but as

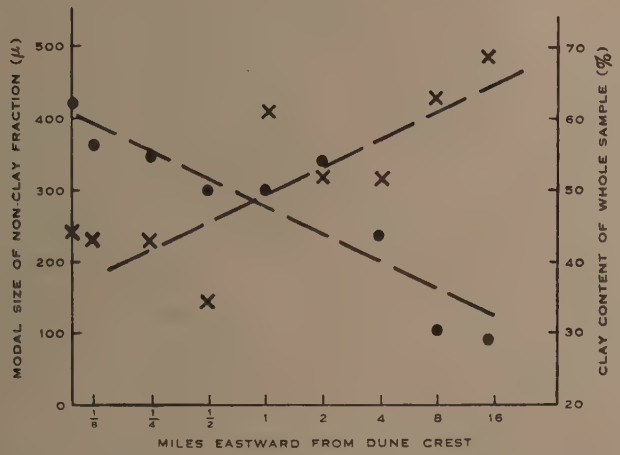

Fig. 6.—Particle size study of sand dune and its related parna sheet: size of non-clay fraction and percentage of clay (whole sample) plotted against distance eastward of dune crest. ● Modal size; × clay content.

probability paper stresses the ends of the summation curves where the particle size distribution data lack accuracy, this suggestion was not investigated further.

In addition to the particle size distribution studies already discussed, other laboratory work was undertaken on the samples to endeavour to characterize parna. Field observations had suggested that the carbonate material in the parna varied from west to east, being highest in the west. This observation, however, was not made any more specific by the results of carbonate determinations on the fine earth and gravel fractions, although there are indications that carbonate in the parna might be as high as 20 per cent. in the west and about 3 per cent. in the east. Carbonates are, however, moderately mobile in soil profiles, and the variable climate already postulated since the parna moved (Butler 1956) must have had a considerable modifying influence.

Determinations of acid-soluble phosphorus and potassium were made on a limited number of samples as a possible indication of the uniformity of the material. The results were not uniform, it was not possible to identify any systematic trend, and the work was not extended further.

An attempt was made to determine the particle size distribution of the material when shaken in a non-aqueous liquid. *n*-Butanol was used but no effective dispersion was obtained, and it is doubtful if any useful data would be obtained, as it is probable that the original clay aggregates have been altered since deposition.

VII. Discussion

A previous publication on parna (Butler 1956) has covered a general appreciation of the material, its possible conditions of formation, and its age. In this paper evidence is brought forward substantiating the main elements requiring proof. It is proposed that the essential proof does not lie in the identification of certain particle size specifications in the parna. These specifications may indeed be quite characteristic and yet justify nothing more than a probability, since the same specifications may occur in riverine and estuarine deposits. The essential proof lies in the uniqueness of the *pattern of variation* of these specifications. In the case of riverine materials the pattern of variation would be related to the river-beds. In the case of parna the variation is in relation to distance leeward of dune zones, and the pattern of variation is not influenced by elevation, since the samples were taken from hills and ridges as well as from sites on the plains overlying a range of riverine materials. These facts point to an aeolian origin for parna. Furthermore the data in Figure 6 show that the sand dune and parna sheet are one system. These two elements are therefore cogenors, i.e. they formed simultaneously and are in fact different facies of one depositional system (wind). Of course it is not proposed that all sand dunes in the region are contemporary with parna sheets; in fact it is known that some were formed in a parnaless period.

Once the main thesis of wind distribution for parna is established, certain other propositions follow. Clay particles would not have been deposited with material of 40-200 μ in size unless in the form of aggregates. The very clayey nature of the material also distinguishes it from loess and supports the proposition that its origin is in weathered soils. These conditions in turn, together with the pattern of distribution (Butler 1956), support the proposition that the climate during deposition was markedly arid.

The trends in mode of particle size (non-clay fraction) and percentage of clay (whole sample) with distance from the sand dune crest are fundamental to the study of parna. The present study, however, must be regarded as only a first approximation to an understanding of the

situation: further work is needed and is planned. The implicit relationship between mode of particle size (non-clay fraction) and percentage of clay* (whole sample) needs further study. It has been shown that the non-clay fraction is highly sorted; but if the sorting of the whole sample is calculated (Trask 1932), values above 1.0 are obtained and the material would be classified as quite unsorted. It is most improbable that the orderliness and sorting apparent in the above studies are the results of chance combinations, and hence it seems that the clay component derives as a secondary effect from the sorting of particles in the 40-200 μ range; that is to say, the clay came into the system as aggregates. This conclusion has already been adduced on other grounds. The particles which travelled in company with the clay aggregates, and which remain after the dispersion of the sample, it is proposed to call *companion sand.* This material can be taken as an indication of the size of the clay aggregates but because of their lower density the aggregates would in fact have been larger than the companion sand. The relationship is indicated by Chepil (1946).

In the saltation zone, or the dune zone, the proportions of sand are variable from one dune system to another even though the pattern of variation within every dune is constant, and as indicated. It seems, therefore, that in this zone, sand of saltation size may have moved without maintaining any fixed relationship to the parna. This suggests bimodal sands: one mode of companion sand and one of saltation sand. The skewness in the size distribution of samples at and near the sand dune zones mentioned in the foregoing data may be evidence of this. However, the present data are insufficient to prove bimodality. One would expect to be able to distinguish between saltation and suspension (dust) materials in terms of the pattern of distribution, the part of the landscape in which they occur, their size; and possibly in their composition. It is likely that these differences between saltation and suspension materials correspond with a size of about 200 μ (Bagnold 1941, p. 6). Dune parna has been mentioned in a previous paper (Butler 1956) and it will be necessary to distinguish between it and the sandy parna on the sand dunes. It may be that there is a similarity of size distributions though a difference in clay content.

Many of the properties of parna other than particle size grading have changed very considerably since deposition. These changes are summed up in the characteristic soil sequences, and may be taken as some indication of the lapse of time and the kind of climate since deposition. By a comparison of the soil on the well-drained site of a younger

* On the basis of the preceding data, % clay $= 126 - 37.5 \log X$, where X is the mode of particle size of the non-clay fraction, expressed in microns. Most of the values fall within $\pm$ 10 per cent. of this relationship, and perhaps it is significant that samples from the dunes fall farthest off. It is not clear at this stage whether this relationship is specific for parna; however, riverine materials plotted on the same axes show a much wider scatter.

parna with that on the Widgelli parna it has been argued (Butler 1956) that the Widgelli period is separated from the present by a long, humid phase, which was, however, broken by a minor arid phase about 5000 years ago. Some further light is thrown on the post-Widgelli climatic conditions by a consideration of the fact that, notwithstanding the highly leached nature of profile A (above) profile E may be calcareous right to the surface. This suggests stagnant, waterlogged conditions on the poorly drained sites—a condition which does not exist under the present climatic regime.

It may be possible to identify the Widgelli parna by the characteristic sequence of soils which occur on it, and this possibility is supported by recent experience on several depositional layers, but it would be sounder to put the final definition in terms of relationship to other depositional layers. Layers both above and below the Widgelli parna have already been mentioned in this and a previous paper (Butler 1956). However, a full elaboration of this aspect of the study must be deferred to a later publication.

The introduction of the study of the Widgelli parna as outlined above opens up new avenues for the pedological study of the region. This aspect need only be touched on briefly now. Widgelli parna is not only a specific parent material (with definable variations) but it is equally specific for the time factor, and also specific for a sequence of climatic phases. The characteristic sequence of soils helps in comprehending the topography-drainage factor. Within these frameworks valid soil comparisons can be made. For example, if it was desired to find what differences in soils could be attributed to differences in climate between, say, Lockhart, N.S.W. (18 in. rainfall), and Swan Hill, Vic. (13 in. rainfall), it would be necessary to exclude soils from the Widgelli parna from the study, since their development is attributable to several prior climatic phases, and not to the present climate. If, alternatively, it was desired to study the difference between soils of Widgelli age attributable to accumulated differences of climate since Widgelli times in the same two localities, it would be necessary to select sites that had all factors common. Thus perhaps sites on "pure" parna may be selected, and topographic-drainage sites corresponding to profile D (Fig. 2). When this is done a certain proportion of clay must be discounted from the Lockhart profile and a certain amount of lime from the Swan Hill profile to compensate for the known variation in parna composition from west to east. Though this study has not been specifically made, it is indicated that the residual difference between the selected Lockhart and the Swan Hill soils is quite small. This suggests that the soil difference here which can be attributed to difference of climate *per se* is very small, and that the greater amount of soil difference between the two localities is due to parent material differences, topographic differences, and others unspecified. Brewer (1954) has made this point as a general proposition for Australia. It seems that until the lack of information on the time and parent material factors

of soils has been made good the significance of climate in soil formation must be largely speculative.

VIII. Acknowledgments

This work has been done with the continual encouragement of Mr. J. K. Taylor, Chief of the Division of Soils, C.S.I.R.O. Many of the authors' colleagues have assisted with discussion of the data and among these should be mentioned Mr. G. A. McIntyre of the Division of Mathematical Statistics, C.S.I.R.O., and Dr. T. J. Marshall of the Division of Soils, C.S.I.R.O. Mr. R. Steel kindly drew the map and diagrams.

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TIME AND TEMPERATURE AS FACTORS IN THE DISPERSION OF SOIL CRUMBS IN WATER

By A. V. Blackmore*

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Summary

A technique is described that enables rates of dispersion of natural soil crumbs to be determined. Mechanical disturbance is kept at a minimum in the method, so that the inherent tendency of the soil to disperse would be the major factor in the result. Limitations of the method are discussed.

The rate and degree of dispersion for six soil materials, representing a range of texture and stability, have been obtained at each of three levels of temperature: 5, 20, and 45°C.

It has been shown that great variation exists between soils in their rate of dispersion, that temperature has a marked effect on these rates, and that for times up to 24 hr the total amount of dispersion achieved is significantly different at each temperature. It is suggested that, in some soils at least, rate of dispersion, and therefore indirectly temperature, are of as much importance in indicating field behaviour as the extent of total dispersion.

I. Introduction

Laboratory studies on the water stability of soil aggregates are undertaken extensively as a means of assessing soil structure and indirectly of understanding the field behaviour of soil. Reviews of this work are given by Baver (1948), Schaller and Stockinger (1953), Low (1954), and others.

When dry crumbs of soil are immersed in water the ensuing activity consists in general of two distinct mechanisms. One is the disruption of the crumb into smaller discrete aggregates, brought about by forces due to swelling and sometimes by pressure of entrapped air (Brewer and Blackmore 1956). The other mechanism is that of the dispersion of the original crumbs or the aggregated products of disruption into primary particles. This is due to the instability under the prevailing conditions of the colloidal material binding these primary particles together to form the structural unit. These mechanisms, although distinct, are not mutually exclusive but may co-exist through a range of degree from 0-100 to 100-0 per cent.

In practice far more attention is given to measuring disruption than dispersion, although this latter property is clearly a more fundamental one and contributes to certain aspects of field soil behaviour independently of those which are a manifestation of swelling and entrapped air.

* Division of Soils, C.S.I.R.O., Canberra.

The present work was undertaken to study the rate of dispersion of natural crumbs, 1.5 to 2.0 mm in size, from a number of soils, and the effect of temperature on this rate and on the degree of dispersion attained. Here then *dispersion* connotes the inherent tendency of the soil to disaggregate, in contrast with the complete dispersion sought, with physical and chemical aids, in studies of particle size distribution.

Laboratory measures of dispersion are based on the presence of excess water, and would be applicable to field conditions at the surface of soils during rain, or for soil material in, or being transported by, rivers. However, for soil materials *in situ,* and away from the soil surface, the conditions of excess water do not exist and, furthermore, there is physical restraint to the movement of soil particles. Under these conditions actual disintegration does not occur, but soil behaviour arising from the same fundamental cause does occur. The lowering cohesion between the clay platelets, which in the unconfined system gives rise to dispersion, in the confined system of the soil *in situ* contributes to plasticity, puddlability, and the like. The laboratory measure of dispersion is therefore a simple measure of a fundamental property in soils which governs several other interesting and important soil states which, being more complex, are not so easily measured.

A conventional method for measuring dispersion (the so-called weak dispersion) has been used by Clarke and Marshall (1947) and others and includes as part of the technique an amount of fairly vigorous shaking of the soil material in water. This shaking enables a reproducible end-point to be reached in a convenient time, but because it is being used partly as a substitute for time and could also cause the dispersion to transcend that which would occur naturally, it renders this technique unsuitable for the present purpose. Accordingly a method has been developed for present use which keeps mechanical disturbance at a minimum.

II. Soil Materials

Structurally undisturbed crumbs of six soil materials have been studied. They are from profiles occurring in the Riverine Plain of south-eastern Australia.

The soils, chosen to represent a range of texture and water stability, are:

(*a*) A grey-yellow clay from a buried old land surface, known as the *Katandra layer* (Butler, unpublished data), which is 10-11 ft below the present surface.

(*b*) A red-brown loam from a depth of 0-4 in. in a marginal red-brown earth profile—*Mundiwa loam* (Smith 1945).

(*c*) A grey light clay from a depth of 0-1 in. in a grey soil of heavy texture profile—*Riverina clay* (Smith 1945; Johnston 1953).

(*d*) A red heavy clay from a depth of 9-13 in. in a red-brown earth profile—*Thulabin loam* (Churchward and Flint 1956).

(*e*) A grey heavy clay from a depth of 2-6 in. in a grey soil of heavy texture profile—*Colchester clay* (van Dijk, unpublished data). This profile is similar to Riverina clay.

(*f*) A red heavy clay (highly calcareous) from a depth of 24-30 in. in a red-brown earth profile—*Ballingall loam* (Taylor and Hooper 1938).

Another aspect of the soil-water relationships of some of these soils has been studied by Brewer and Blackmore (1956).*

III. Method

(*a*) *Physical Data*

(i) *Particle Size Analysis.*—The hydrometer procedure described by Piper (1950) was followed, with the modification of using "Calgon" and caustic soda as dispersing agents.

(ii) *Weak Dispersion.*—A quantity of air-dry aggregates between 2.0 and 0.2 mm in size and equivalent to 62.5 g oven-dry was wet overnight by capillarity, transferred to a 1250 ml sedimentation cylinder of water, and shaken on an end-over-end shaker for 30 min. The percentage of particles of equivalent diameter $< 20\ \mu$ and $< 2\ \mu$ were determined and expressed as a percentage of the total material of these size ranges in the original sample.

(*b*) *Chemical Data*

The methods used were all as described by Piper (1950).

(i) *Soil Reaction.*—Glass electrode method.

(ii) *Total Soluble Salts.*—Specific conductivity method.

(iii) *Chlorides.*—Electrometric titration method.

(*c*) *Dispersion*

A 1.00 g sample of dust-free aggregates 1.5-2.0 mm in size was poured into a 6 by 1 in. glass tube previously filled with distilled water at the required temperature. The apparatus is shown in Figure 1. The tube was covered and maintained at this temperature during the necessary time interval. At the end of the interval a cork slide, 1.4 cm in diameter and 2.6 cm long with wire loop guides and with pin stops 0.5 cm long, was inserted in the tube. The tube was sealed, so as to exclude air bubbles, with a drilled rubber bung, the small hole in the bung being stopped with a wooden peg. The device was inverted, held until the sample had come

* The materials referred to by Brewer and Blackmore as being from Tharbogang loam and Riverina clay profiles are identical with those now considered to be from Ballingall loam and Colchester clay respectively.

clear of the bottom and then returned to its upright position. The turbulence created behind the rising slide and the falling of the aggregates themselves serve to separate the dispersed silt and clay from the remainder of the sample and to suspend them evenly through the water. This cycle of inversion was repeated a further four times in continuous succession, the time for the slide to move from one end of the tube to the other being

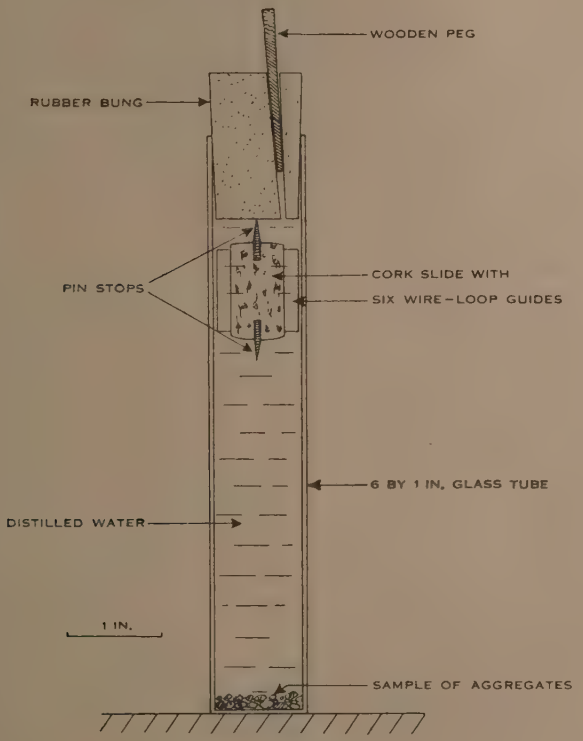

Fig. 1.—Apparatus.

the controlling factor. The bung and slide were then removed and a 5 ml pipette sample taken of the $< 20\ \mu$ material in suspension. The pipetting was done at a depth of 5 cm and at a time appropriate to the temperature of the suspension.

The concentration of the 5 ml aliquot was measured with a spectrophotometer. After diluting (where necessary) and shaking, part of the suspension was placed in a cell 1 cm square and the percentage transmission of 9000 Å light determined. The transmission zone in the cell begins 1.5 cm from the top, and so that sedimentation will not affect the result, the reading must be taken within $\frac{1}{2}$ min of filling the cell. Use of a

calibration curve for each soil enabled concentration to be interpolated directly. The result expresses the amount of $< 20\ \mu$ particles that have dispersed as a percentage of the total quantity of these particles in the initial 1 g sample.

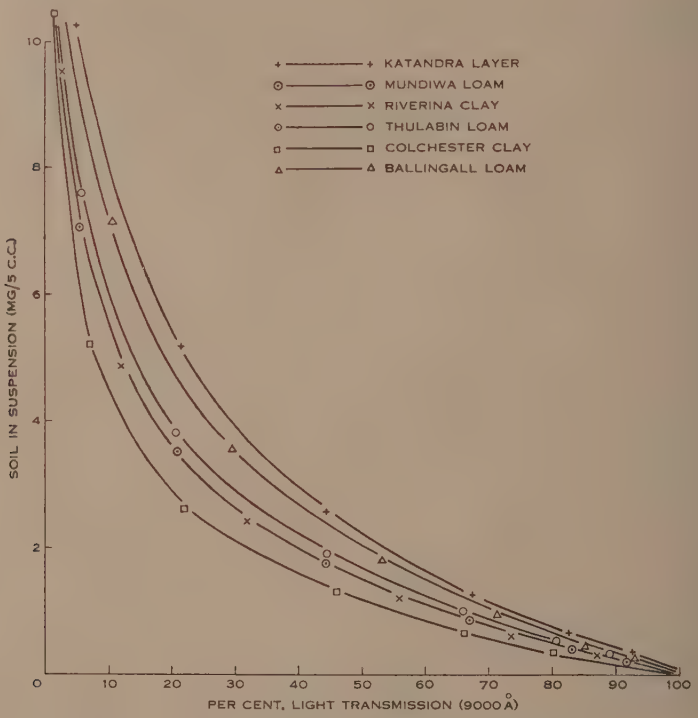

Fig. 2.—Spectrophotometric calibration curves.

IV. Results

The basic physical and chemical data are set out in Table 1.

Gravimetrically checked suspensions were diluted in known proportions to obtain the spectrophotometric calibration curves given in Figure 2. The instrument was set to read 100 per cent. transmission with distilled water in the cell.

Samples of Thulabin loam were used to check whether the number of cycles of inversion was satisfactory, and to find the effect of temperature (through its effect on the physical properties of the water) on the work done by the slide. The crumbs were allowed to rest for 1 hr in water at 20°C and then subjected to from 1 to 9 cycles of inversion. Another set of samples were treated similarly, but after 56 min at 20°C they were cooled rapidly to 5°C before the inversions were carried out. The data

TABLE 1

SAMPLE PROPERTIES

Sample	Chemical Data			Physical Data						
				Particle Size Distribution				Air-dry Moisture Content	Weak Dispersion	
	pH	Total Soluble Solids (%)	Chloride (% NaCl)	Coarse Sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	(g/100 g)	Stability of $< 20\ \mu$ Fraction (%)	Stability of $< 2\ \mu$ Fraction (%)
Katandra layer	7.4	0.055	0.008	11	24	13	52	4.5	12	19
Mundiwa loam	6.9	0.019	0.012	17	47	20	16	1.1	36	56
Riverina clay	7.0	0.021	0.008	18	36	18	28	1.9	57	75
Thulabin loam	7.9	0.039	0.016	1	21	15	63	6.0	20	35
Colchester clay	7.7	0.029	0.008	4	15	11	70	7.2	66	80
Ballingall loam	9.5	0.159	No data	4	37	18	41	6.8	58	75

are plotted in Figure 3, and show that while the amount of breakdown effected by the motion of the slide is quite appreciable the effect of temperature on this aspect alone is quite small.

At three levels of temperature—5, 20, and 45°C—for each soil, dispersion was continued for from 30 sec to 24 hr. Five cycles of inversion were employed throughout to separate the dispersed $< 20\ \mu$ material for

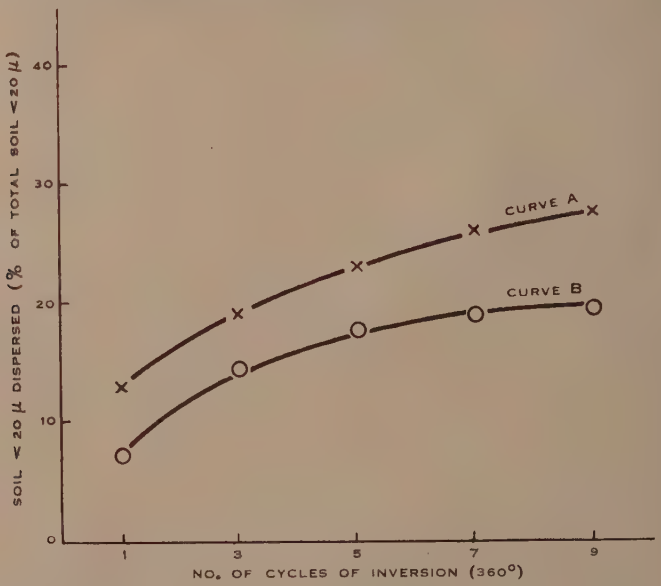

Fig. 3.—Effect of inversions on dispersion and of the temperature at which the inversions are carried out. Curve *A*, sample dispersed for 60 min at 20°C with inversions at this temperature. Curve *B*, sample dispersed for 56 min at 20°C, cooled for 4 min to 5°, and then inverted at 5°.

measurement. The results are given in Figure 4. They show that soils vary greatly in their rates of dispersion and that temperature may profoundly influence these rates.

Throughout the work above a separate sample was used for each single determination of dispersion.

V. Discussion

(*a*) *Method*

The method described for measuring dispersion is an empirical one. The size distribution of the crumbs in the sample will have a direct bearing on the rate of dispersion obtained because dispersion is largely dependent on the area of crumb surface exposed to the water. As a result, varying tendencies to disrupt will also differentially affect dispersion.

The latter factor has been partially overcome by using initially small aggregates. The initial water content and the rate of wetting of the

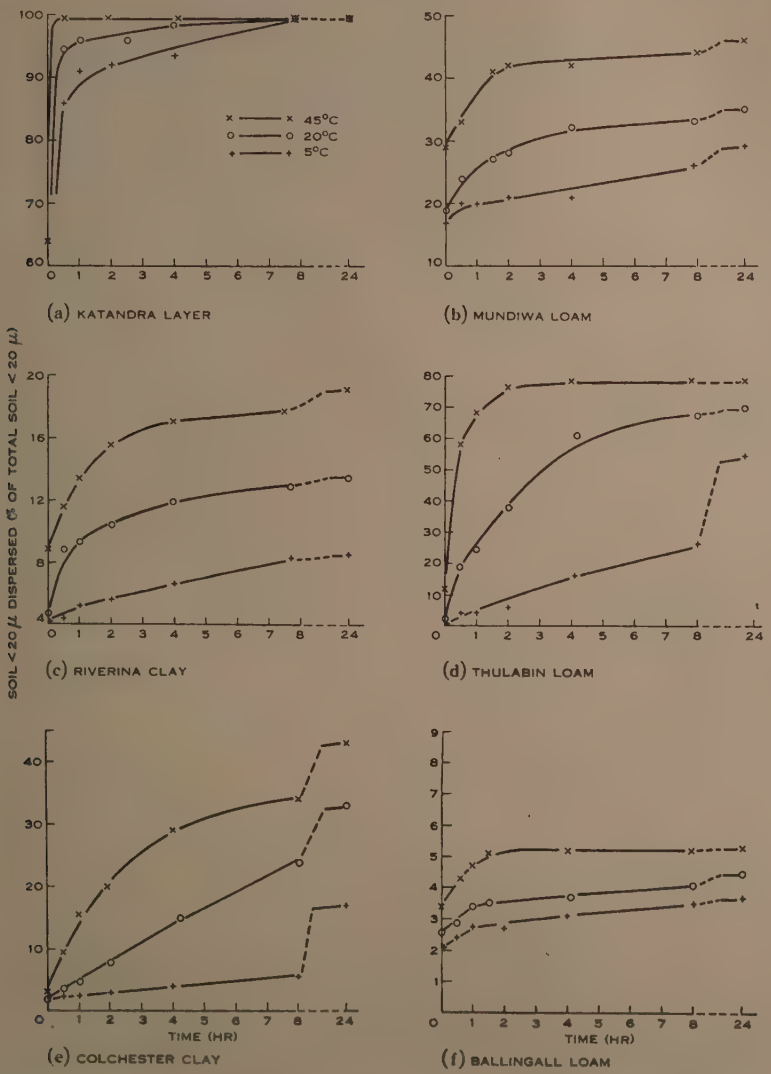

Fig. 4.—Effect of period of immersion in water upon dispersion for six soils at three levels of temperature.

sample may in some types of soil also affect dispersion rates as measured here, but these factors have not been taken into account.

The differences in the calibration curves (Fig. 2) between the soils are due mainly to differences in particle size distribution within the $< 20\ \mu$ fraction. The validity of their use is based on the presumption that the relative composition of the $< 20\ \mu$ particles at various stages of dispersion for any particular soil remains constant. This is undoubtedly not strictly correct but the error introduced would be small compared with the differences in dispersion recorded.

The number of inversions of the tube is also quite arbitrary and was chosen initially by visual means, a minimum number of slide strokes consistent with attaining a uniform suspension being the criterion. The principle of the mixing is the turbulence created behind the rising slide, but the falling aggregates themselves also contribute. As the physical properties of the water change with temperature the effectiveness of the slide might be expected also to vary with temperature. The increasing difference between the two curves of Figure 3 shows that in fact these differences are negligible compared with the differences in rates of dispersion recorded. One basic cause of the separation of the two curves is the reduced rate of dispersion during the 4 min of cooling of the samples represented by the lower curve. This, however, is insufficient to explain the full extent of the separation. The amount of disintegration effected by the slide is apparently quite appreciable and this contributes to the empiricism of the measure. The reproducibility of the technique is of a high order and for the purposes of comparison intended here it is reasonably satisfactory.

In what follows some use is made of data (Table 1) obtained by the conventional weak dispersion method, for purposes of comparison. The cogency of this comparison is reduced somewhat in that this latter technique, because of its excessive shaking, introduces the strength of the wet aggregate as a factor in its final result.

(*b*) *Dispersion*

Dispersion of Katandra material is little affected by temperature (Fig. 4(*a*)). Disaggregation is rapid, being virtually completed within an hour regardless of the temperature. This type of dispersion is responsible for the difficulty in wetting the interior of clods and for the impermeability of this soil. The almost instantaneous dispersion of colloidal particles at the surface prevents further water entry and so restricts the tendency to disrupt that might otherwise be present.

The behaviour of Thulabin loam (Fig. 4(*d*)) contrasts with Katandra material. This soil also has a low level of $< 20\ \mu$ stability, 80 per cent. of its silt and clay being potentially disaggregated, yet its dispersive behaviour is profoundly different. It disperses slowly and is extremely sensitive to temperature. At low levels of temperature it takes more than 24 hr to disperse and after this time there are still significant differences

between the amounts in each of the three cases. At 5°C the soil behaves as a well-structured material, at least for a certain time, while at 45°C its properties are those of Katandra material. Its field reputation as a well-structured B horizon may well arise from this phenomenon. Its structure is preserved at normal temperatures for sufficient time to allow excess water to drain from the profile, thus preventing further dispersion. Here the standard weak dispersion result of 20 per cent. stability is particularly misleading, for with this soil it is apparently the *rate* of dispersion which is the controlling condition.

Colchester clay (Fig. 4(*e*)) exhibits an even slower rate of dispersion than Thulabin loam and a marked temperature effect. Even after 24 hr only one-third as much dispersion occurs at 5° as at 45°C. As with most of the other samples the time-dispersion curve is linear at 5°C, losing this characteristic progressively as the temperature is increased. The intermediate position here of the instability figure (34 per cent.) obtained by the weak dispersion method, with thorough shaking, suggests that the degree of dispersion ultimately obtained is directly related to temperature. Alternatively it is possible that even the overnight wetting by capillarity plus the shaking does not provide sufficient time for complete dispersion to take place; this must depend largely on the initial aggregate size distribution in a material so slow to disperse.

Ballingall loam (Fig. 4(*f*)) is a subplastic clay (Butler 1955) exhibiting extreme dispersive stability. However, it disrupts violently upon immersion and these mild explosions would provide most of the $< 20\ \mu$ material recorded. The violence of the reaction increases and its duration decreases with an increase in temperature, hence the relation between the three curves. The value of 58 per cent. stability obtained with the conventional shaking method is here again misleading.

The surface soil materials Mundiwa loam and Riverina clay also show departure from the behaviour expected on consideration of the conventional weak dispersion results. Mundiwa loam (Fig. 4(*b*)) has a high threshold value of dispersion but thereafter disperses slowly; after 24 hr at 5°C the dispersion is still below the 30-sec value at 45°C. The slow dispersion and large temperature effect confirm that the high initial values of the curves are due to the work done by the slide and not to rapid disintegration. When wetted the aggregates of this soil do not disrupt appreciably but are extremely weak and thus are partially broken up by the motion of the slide. It is this lack of strength, and not necessarily colloid instability, that explains the low stability value (36 per cent.) obtained with the weak dispersion method. At the lower temperatures Mundiwa loam appears a rather stable material, in a purely dispersive sense.

Riverina clay (Fig. 4(*c*)) is extremely slow to disperse at 5°C; after 24 hr it reaches only the 30-min level for 20°C and remains below the 30-sec value for 45°C. The large discrepancy between the weak

dispersion data (57 per cent. stability) and these values is again due to the mechanical action involved with the former.

The results have some significance in the general field of laboratory technique—for example, in the standard Yoder (1936) type of wet sieving procedure for the determination of water stability in the form of the percentage of water-stable aggregates $< 20\ \mu$ in size. With soil materials of the Thulabin loam or Colchester clay type, and with the normal maximum of 1 hr in contact with water plus a light reciprocating motion, a high value for water-stable aggregates would result. If these samples were immersed for 24 hr, permitting a much greater amount of dispersion to occur, the result would be very different. That the initial aggregates are often of at least 5 mm size in this method would enlarge the effect of dispersion rate even more. In such soils this type of measure might well have very little meaning, even as an empirical indicator of soil structure. It may give the size and amount of secondary aggregates, but nothing about their true water stability. Since temperature can radically alter the rate of dispersion, as was shown above, such a wet sieving procedure is subject to this factor also.

Such an argument might easily be extended to any soil material, whether surface or subsurface, whose aggregates in the early stages of dispersion retain sufficient cohesion to withstand the gentle abrasion of the sieving under water.

Low (1954) has reported significant effects of temperature in water-stable aggregate determinations, but Payne (1954), using a surface area method, found only minor effects.

VI. Conclusions

The rate of dispersion of a given size of natural soil crumb varies extensively between soils. The differences in field behaviour of two soils whose ultimate stabilities are comparable may be explained in terms of these contrasts in dispersion rates. Temperature, in significantly affecting the rate and the degree of dispersion attained up to 24 hr, could influence field behaviour and laboratory techniques that are dependent on these properties.

VII. Acknowledgments

The author is indebted to Dr. T. J. Marshall, Division of Soils, C.S.I.R.O., for his helpful advice and criticism during the course of the investigation.

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THE EFFECT OF COLD TREATMENT ON MUTATION IN *DROSOPHILA MELANOGASTER*

By J. M. Rendel* and B. L. Sheldon*

(*Manuscript received June* 11, 1956)

Summary

The effect of cold shocks of various kinds on the mutation rate of *Drosophila* is reported. No effects on mutation rate were found.

I. Introduction

Statements in the literature that cold shocks increase the frequency of lethal mutations in *D. melanogaster* appear to be based on two experiments, one by Birkina (1938), the other by Kerkis (1941). In the first experiment eggs were treated at $-6°$ or $-12°$C for 25-40 min and the number of sex-linked recessive lethals produced was 0.69 per cent., compared to 0.22 per cent. in controls. Hatchability of treated eggs was 4-18 per cent. In the second, adult males were treated at $-5.5°$C for $1\frac{1}{2}$-2 hr, the rate of mutation being 1.42 per cent. as against 0.26 per cent. in controls. Only 0.1 per cent. of adult males remained alive and fertile in this latter experiment.

Since the use of frozen semen in agricultural practice is increasing with the spread of the technique of artificial insemination, and since semen for artificial insemination is normally stored at 4.0°C, it is of importance to know in much greater detail what the relationship is between cold shocks and mutation rate, and whether the rate is ever likely to be so great that it should be taken into account when designing breeding plans to be followed at artificial insemination centres. There appear to be two main questions from the practical point of view. First, is the rate of mutation related to the amount of drop in temperature? Deep-frozen semen is stored at about $-80°$C, but as there is so far no technique of recovering adult male *Drosophila* from this temperature, the best indication of what to expect at this temperature would be given by establishing the trend in mutation rate over temperatures from which *D. melanogaster* can be recovered in a viable condition—that is, somewhere in the region of $-15°$C for adult males and $-30°$C for eggs. The second question is, is the casualty rate brought about by the treatment a better indication of mutation rate than the temperature at which treatment was given? Deep-frozen semen is so treated that some 60 per cent. of sperm remain viable as against 90 per cent. in unfrozen semen; this is an altogether different order of survival rate from that of the flies treated in the mutation trials quoted above. If mortality is a better indication of

* Animal Genetics Section, C.S.I.R.O., University of Sydney.

mutation rate than temperature, then we may expect deep-frozen semen which has been protected from the lethal effects of freezing to have a lower mutation rate than flies which suffered a very heavy mortality and reduction in fertility even though at a higher temperature.

The plan of this experiment was therefore to test the mutagenic effect of various temperatures on *Drosophila* and to apply each temperature for a series of different times in order to vary the mortality rate at each temperature. We hoped to be able to see from this design to what extent mutation rate in the survivors was associated with mortality and to what extent with temperature independent of mortality.

This experiment was not designed to show what effects long storage at −79°C may have on mutation rate. It is unlikely that sperm stored for 5 or 10 years would be much affected by radiation unless exposed to a source much higher than the normal background. If mutation rate could be related to mortality one could get some guide to the effect of cold storage over long periods from the extent to which the sperm survived. For although sperm with severe genetic damage appear to be capable of fertilizing normally there is in general a correlation between mutagenic effects and other damage for any given mutagenic treatment, as one would expect for any mutagenic treatment having effects on structures other than the genetic material. The striking exception is formalin, for which, however, there may be explanations which cannot be discussed here.

II. Material and Methods

The material used was an Oregon-R-C wild type stock of *Drosophila melanogaster*, cold treatment being applied to both adult males and eggs of this stock. The number of recessive sex-linked lethals was used as a measure of mutation rate.

Adult males (up to 3 or 4 days old) were subjected to cold treatment in several different ways, exact details of which will be seen from Table 1 in the following section. At the beginning of the experiment males were placed in empty, stoppered 3 by 1 in. specimen tubes and left either in the main compartment or the freezing compartment of a refrigerator for varying periods of time. In the second series of treatments adult males were placed in 2-c.c. ampoules, which were sealed and put in a small bath of alcohol at room temperature, the temperature of the bath being lowered by a surrounding mixture of dry ice and alcohol until the desired temperature (−15 to −18°C) was reached. The ampoules were then transferred from the bath usually to a constant temperature room at 25°C.

A control was run in conjunction with the treatments to obtain an estimate of the spontaneous mutation rate of untreated males. In addition other recent figures for the spontaneous mutation rate of this stock were already available (Briggs, unpublished data). Except as indicated in Table 1, all test matings of treated or untreated adult males or adults

TABLE 1

SURVIVAL, FERTILITY, AND MUTATION RATES OF ADULT MALES AFTER LOW TEMPERATURE SHOCK

Treatments giving approximately the same mortality have been pooled

	Group	Number Treated	Number of Survivors	Number of Survivors Giving Progeny in F_2	Number of X-Chromosomes Tested	Number of Lethals	Percentage of Lethals	Fiducial Limits (5% level)
(*A*)	Control (Briggs, unpublished data)				2386	5	0.21	
	Control (tested against ClB)				1201	4	0.33	
	Control (tested against Muller-5)				2823	6	0.21	
					6410	15	0.23	0.13-0.39
(*B*)	In refrigerator in vials at:							
	(1) 8°C for 30 min		100%		2576	8	0.31	
	(2) 8°C for 40 hr		100%		583	—	—	
					3159	8	0.25	0.11-0.50
	In freezer (vials) and removed when temperature in vial reached:							
(*C*)	(*a*) −5°C after 22 min	30	30 (100%)	1	589	3	0.51	0.11-1.49
(*D*)	(*b*) −10°C after 27 min	30	5 (17%)		261	—		
(*E*)	(*c*) −15°C after 27 min	30	1 (3%)	5	153	—		
	In alcohol bath (sealed ampoules) lowered from room temperature to:							
(*F*)	(i) −16°C in 27 min	300	145 (50%)	91	2460	7	0.28	0.11-0.59
(*G*)	(ii) −15°C in 25-35 min	1500	304 (20%)	52	2324	4	0.17	0.05-0.44
(*H*)	(iii) −18°C in 32-35 min	1900	106 (6%)	21	1007	4	0.40	0.11-1.02
	In freezer (vials) at −5° to −10°C for 10-12 min							
(*J*)	(tested against ClB)	*c.* 6000	266	93	1608	6		
	(tested against Muller-5)	1700	76	11	506	2		
		7700	342	104	2114	8	0.38	0.16-0.75

from treated eggs were made to a Muller-5 tester stock. The exceptions were test-mated to another ClB stock.

Treatment of eggs was carried out as follows. Large numbers of eggs were collected during a 3-hr period on petri plates of standard corn meal-agar culture medium, and washed off with distilled water. They were then dechorionated by leaving them for 2 min in a 3 per cent. solution of sodium hypochlorite, washed thoroughly with distilled water, and finally suspended in 10 per cent. glycerol. This suspension was transferred to 2-c.c. ampoules, which were sealed and treated similarly to the ampoules containing adult males, except that they were taken down to a lower temperature (−30°C in the alcohol bath) at a slightly quicker rate. Thawing was quickly induced by immersing the ampoules briefly in a water bath at 30°C. The contents of each ampoule were transferred to a standard culture bottle, excess glycerol removed, and the cultures incubated at 25°C. Adults emerging after 10-15 days were mated to Muller-5 males or females for the usual test of mutation rate. About 40 F_1 females were tested from each female egg to be sure of detecting those heterozygous for a lethal before treatment.

III. Results

(a) *Treatment of Adult Males*

The details of the various treatments and their corresponding results are set out in Table 1. A variety of treatments was used in an attempt to find methods which would give repeatable results as far as survival rates were concerned. This was not achieved to any large extent since the mortality under any of the more severe treatments was extremely variable. Thus if several sealed ampoules were kept in an alcohol bath till the temperature reached −15°C after about 30 min, some ampoules would have a survival rate of over 50 per cent., most would be around the 20 per cent. mark, and a few would show less than 10 per cent. survival. The results, however, cover a fair range of temperature treatments and different survival rates, though some treatments, as can be seen from Table 1, are represented by a relatively small number of tested X-chromosomes.

The relationship of fertility of survivors to severity of cold shock can be seen especially from the results of treatment of males in ampoules under the conditions quoted (Table 1). Thus, at a survival rate of 50 per cent., over 60 per cent. (91 out of 145) of survivors were fertile as measured by the number giving progeny to be scored in F_2. When the number of survivors was 20 per cent., about 17 per cent. (52 out of 304) were fertile, and similarly for the lowest survival rate of less than 10 per cent.

The mutation rate, as measured by the percentage of X-chromosomes carrying a recessive lethal, shows no particular trend over the whole

TABLE 2

SUMMARY OF MUTATION RATES FOR ADULT MALES AT DIFFERENT LEVELS OF MORTALITY

Group	Percentage Survival Among Treated Males	Number of X-Chromosomes Tested	Number of Lethals	Percentage of Lethals	Fiducial Limits (5% level)
A	Control	6410	15	0.23	0.13-0.39
B	100%	3159	8	0.25	0.11-0.50
Total control		9569	23	0.24	
C+*F*	50-100%	3049	10	0.33	0.16-0.60
D+*G*	*c.* 20%	2585	4	0.15	0.04-0.40
E+*H*+*J*	10%	3274	12	0.37	0.19-0.64
Total treated		8908	26	0.29	

series of treatments, and in no case is the rate for treated males significantly greater than the figures for the controls (0.23 per cent.). Fiducial limits have been calculated from the table given by Stevens (1942). Treatments giving approximately the same mortality have been pooled and the results are summarized in Table 2, still with no significant differences.

(*b*) *Treatment of Eggs*

The figures given for the number of eggs treated (Table 3) are extremely approximate, but represent a minimum estimate, so that the figures for the percentage hatched are too high. It has been roughly estimated that the normal hatchability for this stock is about 60 per cent. Only very rarely was an adult produced when ampoules of eggs in glycerol were taken down to a bath temperature below −30°C.

The number of chromosomes tested and number of lethals scored (Table 3) are given separately for males and females, and for the two separate attempts at this treatment ((*a*) and (*b*)), all being finally pooled to give an overall estimate of 0.43 per cent. (21 out of 4929 X-chromosomes tested). There appears to be some indication here of a slight increase in the mutation rate under cold treatment but it is not large and in these data not significantly different from the control in Table 1.

Three adult females have been obtained as a result of treating eggs by the same methods, but taking the temperature down to −79°C and leaving them at that temperature for about 18 hr. These three adults were the sole survivors of some tens of thousands of eggs subjected to this treatment. Two out of the three were tested for mutation rate against Muller-5 stock. 399 X-chromosomes were tested and only one recessive lethal was obtained. This, of course, is very slight, if not negligible, evidence on whether the mutation rate is increased at all by such an extreme treatment. Unfortunately no further adults have been obtained after treatment at −79°C, and until this can be repeated the possibility of contamination cannot be ruled out.

IV. Discussion

Since we have been unable to produce any significant increase in mutation rate by cold treatment, the original intention of the experiment has had to be abandoned. Our results indicate that if there is any effect of cold on mutation rate it must be small. Since an increase was found by Kerkis after treatment which resulted in a very high mortality it must be assumed that some increase in mutation may be expected. But the work of Nilan (1954) on deep freezing barley seeds, in which a temperature of −80°C produced no increase in mutation rate or frequency of chromosomal abnormalities, combined with the findings reported here

TABLE 3

MUTATION RATE AFTER LOW TEMPERATURE TREATMENT OF *DROSOPHILA* EGGS

Treatment	Number Treated	Number of Adults Produced	Number Giving Progeny in F_2	Number of X-Chromosomes Tested	No. of Lethals	Percentage of Lethals	Fiducial Limits (5% level)
Eggs in glycerol taken from room temperature down to −30°C in 35-40 min	(*a*) 5000	27♂♂, 30♀♀ (1.2%)	22♂♂, 21♀♀	From ♂♂ 1023	3	0.29	
				From ♀♀ 1924	8	0.42	
	(*b*) 4000	28♂♂, 15♀♀ (1.1%)	23♂♂, 10♀♀	From ♂♂ 1339	6	0.45	
				From ♀♀ 643	4	0.62	
	(*a*) + (*b*) 9000	55♂♂, 45♀♀ (1.1%)	45♂♂, 31♀♀	From ♂♂ 2362	9	0.38	0.17-0.72
				From ♀♀ 2567	12	0.47	0.24-0.82
				4929	21	0.43	0.27-0.65

indicate that the risk of increasing mutation by an amount which is dangerously high is small and not such as to deter practitioners from the use of deep-frozen semen with artificial insemination.

V. Acknowledgment

We are indebted to Mr. A. W. Blackshaw of the Department of Veterinary Physiology, University of Sydney, for assistance with the freezing technique, which was adapted from that evolved by Professor C. W. Emmens of the same Department for the freezing of semen.

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THE DEVELOPMENT OF THE TESTIS, EPIDIDYMIS, AND PENIS IN THE YOUNG MERINO RAM

By R. H. Watson,* C. S. Sapsford,† and I. McCance*

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Summary

Observations were made on 110 Merino rams aged between 1 and 64 weeks. They comprised four groups, the animals of which experienced different rates of growth.

The weight of the testes at any one age varied widely. It was closely related to body weight. This relation was similar in all groups. As body weight increased from 23 to 27 kg, the relative increase in the weight of the testes was much greater than that at higher or lower body weight.

The general histological characteristics of the testes were closely related to their weight, and, like it, to body weight. The tubules were present as solid or but slightly vacuolated cords in all animals which weighed less than 21 kg. Spermatozoa were not seen in the lumen of the tubules in any animal which weighed less than 27 kg, and they were present in all but three of those which weighed more than 28 kg. The relative cross-sectional area of the seminiferous tubules increased directly with the relative increase in the weight of the testes until the testes weighed at least 300 g.

The weight of the epididymides was more closely related to the weight of the testes than to age or body weight. Its relation to the weight of the testes varied in the different groups.

Irrespective of the age at which the particular body weight was attained, little separation of the prepuce from the penis had occurred in animals which were lighter than 18 kg, and separation was more or less complete in most animals which were heavier than 27 kg.

I. Introduction

Phillips and Andrews (1936) and Carmon and Green (1952) made a small number of observations on the development of the testes in Shropshire, Southdown, and Hampshire rams. Carmon and Green made some observations on the development of the epididymides also. Johnstone (1948) described the post-natal development of the penis in Merino rams; and Wiggens and Terrill (1953) determined the influence of various factors upon it in Columbia, Targhee, and Rambouillet rams. Of these various observations, only those of Johnstone refer to Merino rams.

Merino ram lambs became available from matings which were undertaken to provide material for another study. The opportunity was taken to use these lambs to obtain information on the development of the testes and epididymides, and to extend the available information on the develop-

* Division of Animal Health and Production, C.S.I.R.O., Parkville, Vic.

† Department of Veterinary Science, University of Sydney.

ment of the penis, in animals of this breed. Details of the observations are presented in the present paper.

While this paper was in preparation, Dun (1955) published some data on the development of the testes and penis in Merino rams. However, his observations were more limited in scope than those presented here.

II. Material and Methods

(*a*) *Animals*

The ram lambs were the progeny of medium fine-woolled Merino ewes and Peppin strain Merino rams from the Riverina district of New South Wales. Most of them derived from the three lambings which are described by Watson (1953). The lambings took place at 4-monthly intervals from October 1947. A few were born 1 to 4 months later from a further mating of some of the same ewes and rams. The animals thus fall into four groups according to when they were born. All derived from single births.

(*b*) *Treatment of Animals*

The animals of groups 1-3 were run throughout on improved pasture at the Field Station, C.S.I.R.O., Tooradin, Vic. Those animals of group 4 from which the testes had not been collected were transferred to irrigated pasture at the Field Station, C.S.I.R.O., Werribee, Vic., in mid December.

The animals were tailed when they were 3-4 weeks old, and they were weaned when they were 13-15 weeks old. All were vaccinated with an alum-precipitated enterotoxaemia vaccine at tailing, and those of groups 1-3 were vaccinated with an attenuated virus of contagious pustular dermatitis at 7-47 days of age. In all other respects treatment followed usual husbandry practice. All animals were weighed direct from pasture at intervals which in most instances were 1-2 weeks, but occasionally were 3-7 weeks.

(*c*) *Feed Conditions*

At both Field Stations most of the rain falls during the autumn, winter, and spring months. Summer conditions are dry and warm with occasional hot days. Whereas at the Tooradin Field Station the pasture dries off during the summer months, and remains dry until the autumn rains, the irrigated pasture at the Werribee Field Station continues to grow throughout the summer months, and does not dry off.

Some oaten chaff was supplied as supplementary feed during the autumn of 1948, and grass hay was supplied during the late summer and autumn of 1949 to the animals at the Tooradin Field Station.

(*d*) *Collection and Treatment of Testes and Epididymides*

Initially in young lambs the testes were collected by removal of the distal half of the scrotum with a scalpel, snipping the tunic of each testis

in turn, extruding the testis through the snipped tunic, clamping off the cord with artery forceps, and cutting it with a scalpel. Later in young lambs the whole scrotum was clamped in the jaws of a Burdizzo castrator, and the scrotum and testes distal to the castrator were removed in one piece with a scalpel. In older animals the scrotum was too large to include in the castrator at one bite. Initially in such instances, the castrator was applied to the left half of the scrotum and the left cord, then to the right half and the right cord. While it was still applied, the right half was severed by a cut along the distal edge of the castrator which was then removed and re-applied to the left half of the scrotum, and the removal of the scrotum completed. Marked injection of the left testis was noted in two animals following this treatment, probably as the result of venous but not arterial occlusion at the first application of the castrator. Subsequently each half of the scrotum was severed immediately the castrator was applied.

Immediately following collection, the epididymis was dissected off each testis in turn, the cord was trimmed off flush with the testis with a razor blade, and each testis was weighed. In many instances each epididymis was also weighed.

After weighing, each testis was cut with a razor blade along a plane through its greatest circumference. A piece of tissue 2-3 mm thick was taken from the margin to the centre of the testis in the middle of this plane. It was laid flat on thin cardboard, fixed in 10 per cent. formalin saturated with mercuric chloride for 4 hr, transferred to saturated mercuric chloride for 12-18 hr, washed in running water for 24 hr, and taken into 70 per cent. alcohol which contained a few drops of iodine. It was stored in this solution for varying periods before embedding.

In many instances, too, a small piece of tissue from the epididymis was treated in the same manner. In these instances a smear was also taken of the tissue fluid at the cut end of the head of the epididymis.

(*e*) *Staining and Examination of Tissue from the Testes*

The fixed tissues were embedded in paraffin, and sections 8 μ in thickness were stained with haematoxylin and eosin. The magnified images of 30 approximately circular tubules from one testis of each animal were traced in pencil on cards. Two measurements, approximately at right angles to one another, were made subsequently of the diameter of the traced image of each tubule. Measurements in which the ratio of the two diameters exceeded 1.2 were discarded. The mean diameter of the tubules was obtained from the other measurements by the application of the appropriate conversion factor to their mean, and the mean cross-sectional area was obtained by calculation.

(*f*) *Examination of the Penis and Prepuce*

The prepuce and penis were everted, and the degree to which the prepuce was separated from the penis was noted.

(*g*) *Allotment of Animals to Groups for Castration*

In general, in allotment of the available animals for castration at the various ages they were treated in batches in the order in which they were born, and one animal from each batch was allotted for castration at each different age. All animals did not have equal opportunity of being selected for castration at all ages, but there was no systematic bias in the selection.

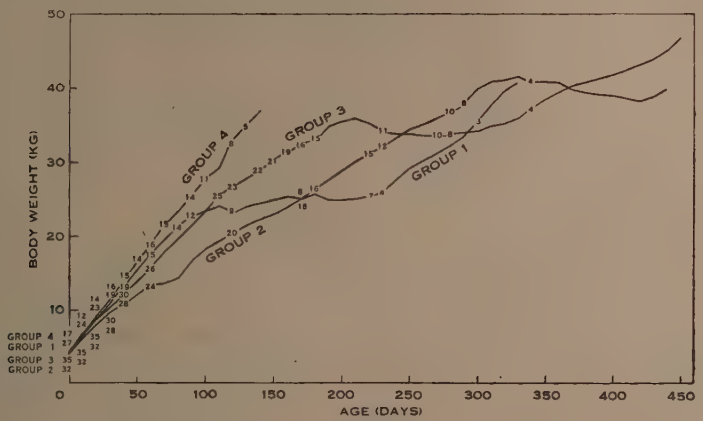

Fig. 1.—Course of growth of the animals of the different groups. The numbers in the figure refer to the number of contributors to each mean. They are omitted when this is unchanged. Their position is of no significance before 40 days.

III. Results

(*a*) *Changes in the Body Weight*

The mean growth curves of the various groups are set out in Figure 1. Each of the groups is characterized by a somewhat higher rate of increase in weight during the suckling period than after weaning, except the animals of group 4. However, the rate of increase was somewhat lower in the animals of group 2, which were born in the summer, than in those of groups 1, 3, and 4, which were born in the spring and the winter. The animals of group 4 continued to increase at a high rate after being weaned onto irrigated pasture during the summer. Those of the other groups exhibited a moderate rate of increase after weaning except when the feed was dry during the summer months, when little or no change in weight occurred. This period when the weight was static occurred immediately after weaning in the animals of group 1, 3-4 months later in the animals of group 3, and 3-4 months later still in the animals of group 2. The mean weight of the animals at birth was closely similar in all groups, but as a result of the differences in the rate of growth, the mean weight of the animals of the several groups at any subsequent age varied widely.

(*b*) *Development of the Testes*

(i) *Increase in Weight.*—The weight of the testes is plotted against the age of the animal at the time of castration and against its body weight in Figure 2, and the logarithm of the weight of the testes (log *TW*) is

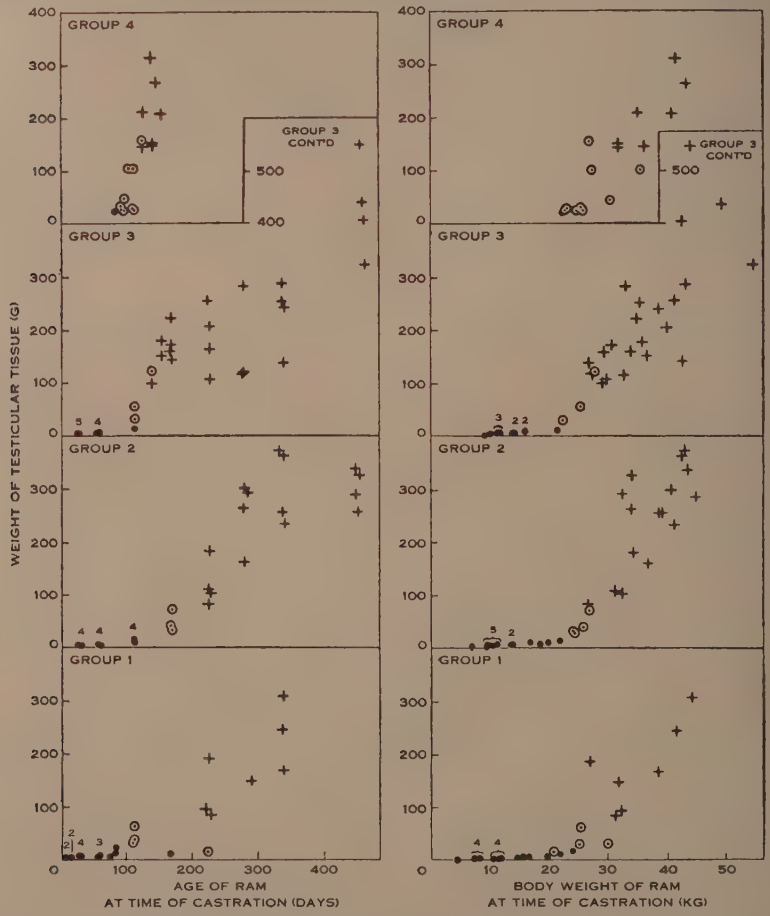
GROUP 4
GROUP 3 CONT'D
GROUP 3
GROUP 2
GROUP 1
WEIGHT OF TESTICULAR TISSUE (G)
AGE OF RAM AT TIME OF CASTRATION (DAYS)
BODY WEIGHT OF RAM AT TIME OF CASTRATION (KG)

Fig. 2.—Relation of the weight of the testes to the age of the animal and to its body weight; arithmetic scale.

● Seminiferous tubules present as solid cords only.

◉ Most or all tubules exhibit a lumen.

+ Spermatozoa present in the lumens of some or all tubules.

plotted against the logarithm of the body weight (log *BW*) in Figure 3. In any one group there was a general increase in the weight of the testes with the age of the animal at the time of castration, but the weight of the testes at any one age varied widely in the different groups. A much

closer relationship, and one which was similar in all groups, existed between the weight of the testes and the body weight of the animal at the time of castration. It is characterized by a slow increase in the weight of the testes until the body weight is 20 kg or more, and a much steeper

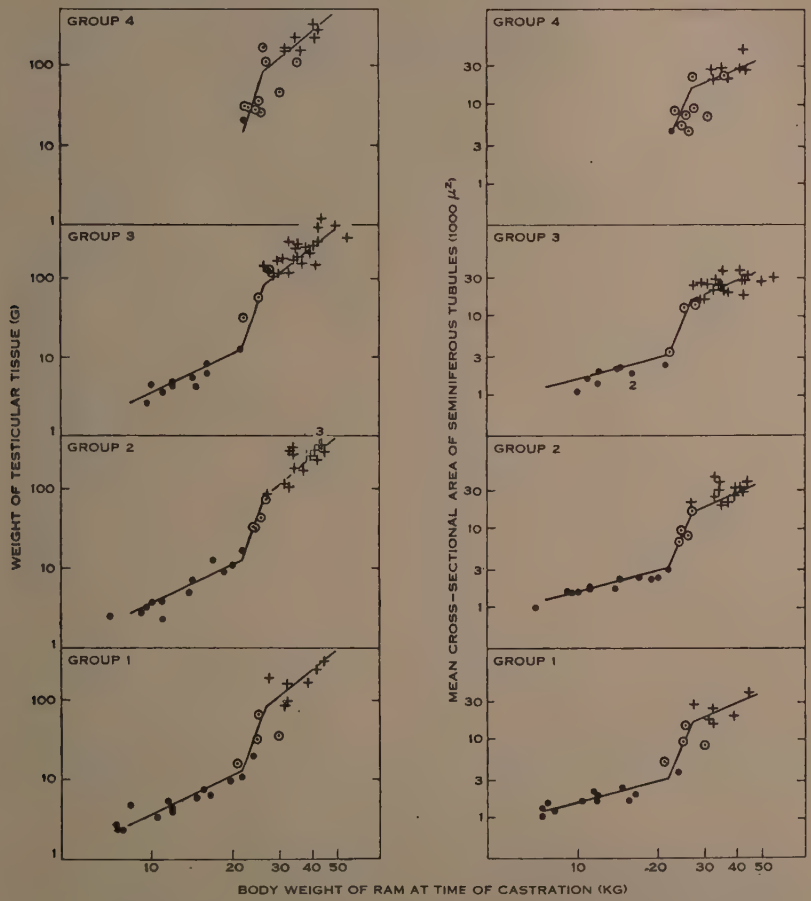
GROUP 4
GROUP 3
GROUP 2
GROUP 1
WEIGHT OF TESTICULAR TISSUE (G)
MEAN CROSS-SECTIONAL AREA OF SEMINIFEROUS TUBULES (1000 μ²)
BODY WEIGHT OF RAM AT TIME OF CASTRATION (KG)

Fig. 3.—Relation of the weight of the testes and the mean cross-sectional area of the seminiferous tubules to body weight; logarithmic scale. The lines are those defined by the constants set out in Table 1. The symbols ●, ◉, + indicate the stage of development of the tubules as in Fig. 2.

increase thereafter. The weight of the testes certainly continued to increase with the body weight until the body weight was 45 kg. The data do not provide evidence of the nature of the relationship in animals with a body weight greater than 45 kg.

As the body weight increased from 23 to 27 kg, the relative increase in the weight of the testes was much greater than that at either the higher or the lower body weights. On treating the data separately within each of these three classes of body weight, as suggested by Brody (1945, p. 610), log *TW* within each of them was found to be related in linear fashion to log *BW*. The values of the constants of the regression lines

$$\log TW(\mathrm{g}) = b \log BW(\mathrm{kg}) + \log a,$$

which were fitted to the data of the three classes, are set out in Table 1

TABLE 1

CONSTANTS OF REGRESSIONS ON THE LOGARITHM OF THE BODY WEIGHT OF THE LOGARITHM OF THE WEIGHT OF THE TESTES AND THE LOGARITHM OF THE MEAN CROSS-SECTIONAL AREA OF THE SEMINIFEROUS TUBULES

Body Weight Class	Number of Animals	Log a*	Regression Coefficient (b)*	Standard Error of b*	Points of Intersection
Log_{10} TW (g) *on* log_{10} BW (kg)					
Up to 22.2 kg	38	−1.01	1.58	0.15	
					21.8 kg
22.7 to 27.2 kg	20	−11.09	9.12	1.26	
					26.8 kg
Above 27.7 kg	52	−1.84	2.64	0.33	
Log_{10} MTA (μ^2) *on* log_{10} BW (kg)					
Up to 22.2 kg	37	+2.03	0.87	0.11	
					21.8 kg
22.7 to 27.2 kg	17	−9.77	7.88	2.19	
					27.2 kg
Above 27.7 kg	46	+1.91	1.30	0.31	

* In the equation $\log Y = b \log X + \log a$.

along with the points of intersection of the lines. The regressions within these three classes account for 96 per cent. of the variation in the weight of the testes.

(ii) *Growth of the Seminiferous Tubules.*—The mean cross-sectional area of the tubules is plotted against the age of the animal at the time of castration, against its body weight, and against the weight of the testes in Figure 4, and the logarithm of the mean cross-sectional area of the tubules (log *MTA*) is plotted against log *BW* in Figure 3. The general state of development of the tubules in each animal is indicated in the various figures; and the curve which relates mean cross-sectional area of the tubules with the weight of the testes is also included in Figure 4.

(1) *Changes in Morphology.*—A detailed description of the cytological changes by one of the authors (C.S.S.) will be published elsewhere. Attention is confined here to the general histological changes which were observed in the development of the tubules.

In the youngest animals the future tubules were seen as tortuous solid cords, in which both distinct cells and a cytoplasmic syncytium could be recognized. The cells were of two types: some with rather large nuclei which were located in the centre of the cord at irregular intervals, and others, more numerous, with smaller nuclei which were located around the periphery of the cord. The cytoplasm of these cells extended towards

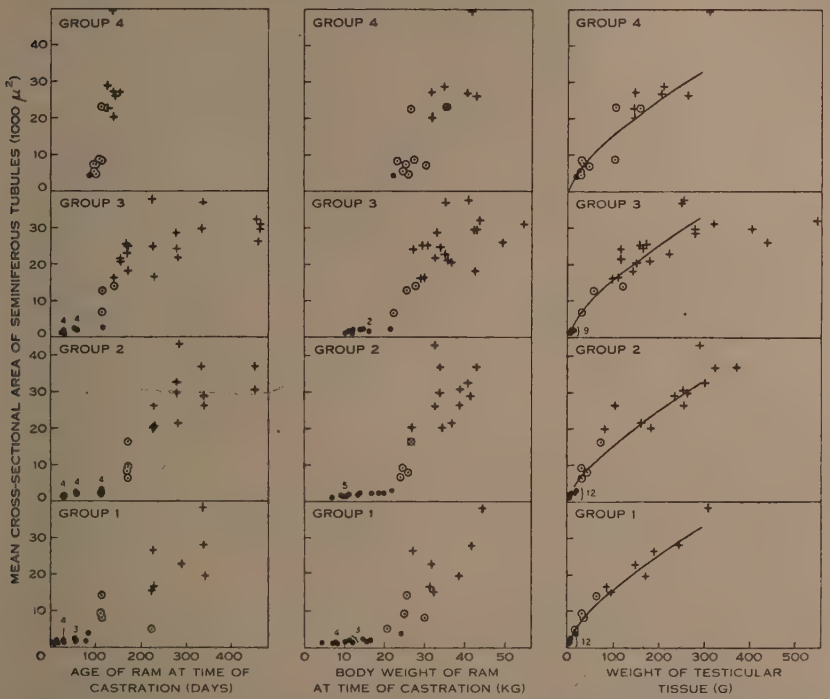
GROUP 4
GROUP 3
GROUP 2
GROUP 1
MEAN CROSS-SECTIONAL AREA OF SEMINIFEROUS TUBULES (1000 μ²)
AGE OF RAM AT TIME OF CASTRATION (DAYS)
BODY WEIGHT OF RAM AT TIME OF CASTRATION (KG)
WEIGHT OF TESTICULAR TISSUE (G)

Fig. 4.—Relation of the mean cross-sectional area of the tubules to the age of the animal, its body weight, and the weight of its testes; arithmetic scale. The curves shown all correspond to the equation

$$\log_{10} MTA \ (\mu^2) = 2.791 + 0.700 \log_{10} TW \ (g).$$

The symbols ●, ◉, + indicate the stage of development of the tubules as in Fig. 2.

the centre of the cord. The nuclei of the syncytium were also peripherally placed, and its cytoplasm extended into the centre of the cord wherever the cells were absent. It will subsequently be shown that the syncytium was a source of Sertoli cells, whereas the cells with the smaller nuclei differentiated into spermatogonia, from which were derived the various cells concerned in the process of spermatogenesis.

The first obvious change in the cords in the post-natal period was the formation of a lumen, accomplished by vacuolization of the cytoplasm

of the syncytium. The formation of a distinct lumen was rapidly followed by, or in some instances was coincident with, the appearance of primary spermatocytes. In a few instances tubules in different stages of development were observed in the one testis, but generally tubules in one or the other stage predominated. After a further interval of time, secondary spermatocytes and later spermatids were observed. The appearance in the tubules of spermatozoa, and their passage to the rete testis and ultimately to the epididymis, completed the development of the tubules.

TABLE 2

AGE AND BODY WEIGHT OF THE ANIMALS IN WHICH DEVELOPMENT OF THE TESTES WAS UNUSUAL

Type of Unusual Development	Group to which Animal Belonged	Age of Animal (days)	Body Weight of Animal (kg)
Body weight >28 kg; sperm not seen in seminiferous tubules	1	112	30
	4	99	30*
	4	112	35
Sperm present in tubules; not seen in epididymis	2	225	31*
	2	225	27
	3	139	29
	4	126	36
Sperm not seen in tubules; occasional sperm in epididymal smear	2	170	24

* The fleece was wet following rain. It probably contained 0.5-1 kg more water than usual.

The general histological characteristics varied widely at any one age, but they were closely related to the weight of the testes, and, like it, to the body weight of the animal at the time of castration. The tubules were present as solid or but slightly vacuolated cords in all animals which weighed less than 21 kg. Spermatozoa were not noted in the lumen of the tubules in any of the animals which weighed less than 27 kg, and they were noted in all but three of those which weighed more than 28 kg. None of these latter three animals was more than 112 days old. Details of their age and body weight are set out in Table 2.

(2) *Changes in Size.*—The mean cross-sectional area of the tubules also increased generally with the age of the animal, but it too was more closely related to both the body weight of the animal at the time of castration and the weight of the testes. In the analysis of the multiple regression of log *MTA* on log *TW*, log *BW*, and age, the variation in the weight of the testes accounted for 96 per cent. of the variation in the mean cross-sectional area of the tubules. The relationship between these two variables was similar in all groups. It is characterized by an increase at a gradually declining rate in the cross-sectional area of the tubules until

the weight of the testes is at least 300-400 g. The testes weighed more than 400 g in only three animals. The cross-sectional area of the tubules in these three animals suggests that the tubules may have ceased to grow in this dimension in these heavier testes.

Log *MTA* is related in a linear fashion to log *TW* by a regression line which is described by the equation

$$\log_{10} MTA\,(\mu^2) = 0.700 \log_{10} TW\,(\mathrm{g}) + 2.791.$$

The standard error of the regression coefficient is 0.013.

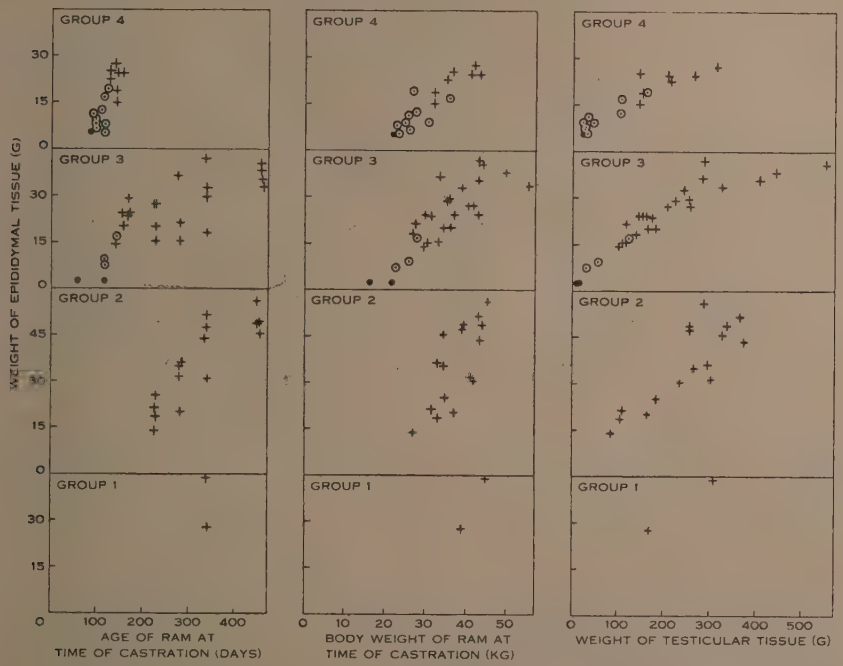
GROUP 4
GROUP 3
GROUP 2
GROUP 1
WEIGHT OF EPIDIDYMAL TISSUE (G)
AGE OF RAM AT TIME OF CASTRATION (DAYS)
BODY WEIGHT OF RAM AT TIME OF CASTRATION (KG)
WEIGHT OF TESTICULAR TISSUE (G)

Fig. 5.—Relation of the weight of the epididymides to the age of the animal, its body weight, and the weight of its testes. The symbols ●, ◉, + indicate the stage of development of the tubules as in Fig. 2.

No doubt as a result of the close relationship between the weight of the testes and body weight, the relative increase in the cross-sectional area of the tubules, as the body weight increased from 23 to 27 kg, was much greater than that at either higher or lower body weights. Lines fitted by the method of least squares to the data within each of these three classes are defined in Table 1 and included in Figure 3. They follow very closely the regression lines which were fitted to the data relating log *TW* and log *BW*.

(c) *Development of the Epididymides*

The epididymides were weighed in some only of the animals. For these animals, the weight of the epididymides is plotted in relation to age at the time of castration, to body weight, and to the weight of the testes in Figure 5.

As with the weight of the testes, the weight of the epididymides exhibited a general increase with age at the time of castration in the animals of any one of the groups, but the weight of the epididymides at any one age varied widely. A somewhat closer relationship existed between the weight of the epididymides and the body weight at the time

TABLE 3

REGRESSION OF THE LOGARITHM OF THE WEIGHT (G) OF THE EPIDIDYMIDES ON THE LOGARITHM OF THE WEIGHT (G) OF THE TESTES

Group	Age of Animals at Castration (days)	Number of Animals	Constant Term (log a)*	Regression Coefficient (b)*	Standard Error of b	Residual Mean Square
2	225-451	16	−0.373	0.807	0.100	0.00635
3	224-461	15	−0.024	0.620	0.073	0.00365
	57-170	12	−0.311	0.756	0.038	0.00359
4	84-154	17	0.071	0.552	0.043	0.00492

Comparison of Regression Coefficients

Groups Compared	F	Degrees of Freedom	Level of P
Group 2 *v.* group 3 (older)	2.28	1, 27	>0.05
Group 3 (older) *v.* group 3 (younger)	2.74	1, 23	>0.05
Group 3 (younger) *v.* group 4	12.3	1, 25	<0.01

* As in Table 1.

of castration. A still closer relationship, indeed a very close one, existed between the weight of the epididymides and the weight of the testes. Analysis of the multiple regression of the logarithm of the weight of the epididymides (log *EW*) on log *TW*, log *BW*, and age showed that the variation in log *EW* was closely associated with that in log *TW*. The regression on log *TW* accounted for 92 per cent. of the variation in log *EW* within groups.

The coefficients of the regression of log *EW* on log *TW* in the data from animals of similar age in the different groups are set out in Table 3. Relative to the increase in weight of the testes, the epididymides increased in weight at a constant rate within each of the groups, at least until the weight of the testes was of the order of 300 g. However, the rate of increase in the animals of group 4 differed significantly from that in the animals of groups 2 and 3.

Insufficient data are available to define the rate of increase in weight of the epididymides relative to that of the testes in animals with testes heavier than 300 g. However, in the three animals of this class in group 3, there was certainly little further increase in the weight of the epididymides. Insufficient data are available, too, to determine whether the relative increase in the weight of the epididymides as the body weight

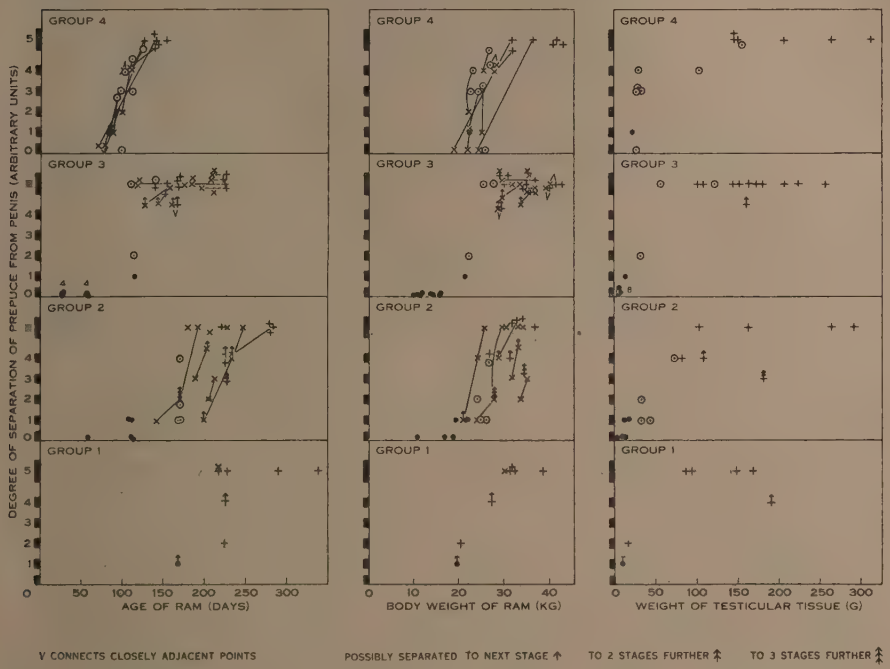
GROUP 4
GROUP 3
GROUP 2
GROUP 1
DEGREE OF SEPARATION OF PREPUCE FROM PENIS (ARBITRARY UNITS)
AGE OF RAM (DAYS)
BODY WEIGHT OF RAM (KG)
WEIGHT OF TESTICULAR TISSUE (G)
V CONNECTS CLOSELY ADJACENT POINTS
POSSIBLY SEPARATED TO NEXT STAGE
TO 2 STAGES FURTHER
TO 3 STAGES FURTHER

Fig. 6.—Degree of separation of the prepuce from the penis, in relation to the age of the animal, its body weight, and the weight of its testes. The symbols ●, ⊙, + indicate the stage of development of the tubules as in Fig. 2. × indicates that the observation was made on a day other than that of castration. Connected points refer to observations on the same animal. Arrowheads indicate that development may have advanced further than the point plotted.

increased from 23 to 27 kg was greater than that at other body weights. However, it must be assumed that this would be so in view of the close relationship between log *EW* and log *TW*.

In all except four of the animals in which spermatozoa were present in the seminiferous tubules, spermatozoa were also present in the epididymis. The four animals in which this did not occur were distributed in three different groups. Details of their age and body weight are included in Table 2. The animal or animals in each group were among the youngest in which spermatozoa were present in the seminiferous

tubules. A very few spermatozoa were also present in a smear from the epididymis of one animal in which spermatozoa were not seen in the seminiferous tubules.

(*d*) *Development of the Penis*

The degree to which the prepuce was separated from the penis was noted in most of the animals of groups 2, 3, and 4, and in a few of the animals which were castrated latest in group 1. In most instances one examination only was made, immediately before the animal was castrated; in a few, one or two earlier examinations were also made. The results are plotted against the weight of the testes, against the body weight of the animal, and against the age of the animal in Figure 6.

The data do not lend themselves to precise statistical analysis. Nevertheless it is clear that the separation of the prepuce from the penis is also much more closely related to the weight of the testes, and to the body weight of the animal, than to the age of the animal. The degree of separation was much the same in all groups in animals of similar body weight, and in animals with a similar weight of testicular tissue. Little separation had occurred in animals which weighed less than 18 kg, and separation was more or less complete in most animals which weighed more than 27 kg. Again, separation had commenced in most animals in which the testes weighed more than 15 g, and in all in which they weighed more than 25 g, and it was well advanced in all animals in which the testes weighed more than 50 g. On the other hand, the degree of separation at any age varied in the animals of the different groups. Separation was complete in all animals of groups 3 and 4 which were older than 100 days, but it was not complete in a number of animals between 140 and 225 days in group 2. Nevertheless the results are suggestive of some influence of age on the course of separation. Thus in the two animals of group 1 which were light in weight, and which had very small testes at 168 and 224 days of age respectively, separation was further advanced than in animals which had achieved similar body weight and similar weights of testicular tissue at earlier ages.

IV. Discussion

The animals used in the present observations were the progeny of ewes selected at random from one lot, and of rams from an even line in another flock. All received the checks to growth of tailing, vaccination, and weaning at much the same age. However, the animals of the different groups were born at different times of the year, and varied widely in body weight at any one age according to the seasonal conditions—mainly nutritional, but also those incidental to prevailing weather—which they had experienced to that age.

Hauser, Dickerson, and Mayer (1952) found in boars that testicular development was more closely associated with body size than with age.

A closer association of testicular development with body weight than with age is particularly evident in the present series of observations as a result of the varied rates of growth of the animals in the different groups.

Whether in the adult animal the weight of the testes continues to increase with the body weight cannot be decided from the present data. Even the oldest animals in the present study were not fully grown. Only the two heaviest animals of group 3 had attained a body weight greater than 45 kg. The mean body weight under similar conditions of the ewes and rams from which they were bred was rarely, if ever, less than 40 and 60 kg respectively.

Whatever the relationship in the adult animal, the growth of the testes relative to that of the body as a whole followed a sigmoid course in the data which were collected. A sigmoid course in the growth of the testes relative to that of the body as a whole is evident also in the data in guinea-pigs, rabbits, pigs, and monkeys of Bessesen and Carlson (1923), Kibler, Bergman, and Turner (1943), Phillips and Zeller (1943), and van Wagenen and Simpson (1954), although Kibler, Bergman, and Turner (1943) fit a straight line to their data. There is slight evidence of a sigmoid relationship, too, in the data of Hooker (1944) on dairy bulls. However, the relationship is certainly not always sigmoid in nature: it is linear throughout the whole range of the body weights, 10-30 kg and 14-45 kg, respectively, of the Southdown and Hampshire rams studied by Carmon and Green (1952); it is also linear up to body weights of 600 g in the guinea-pigs of Mixner, Bergman, and Turner (1943); and in the various strains of pigs studied by Hauser, Dickerson, and Mayer (1952), it varies from one with a gradually increasing slope, through a linear one, to one with a sharply decreasing slope. The significance of these variations in the relationship is not evident.

In these Merino rams, growth of the seminiferous tubules in cross-sectional area certainly contributed to the growth of the testicular tissue until it attained a weight of some 300 g. Up to this weight at least, too, the relative contribution of growth from this source to total growth was constant. Either, then, the relative contribution of growth from other sources—the length of the tubules and the amount of interstitial tissue—must also have remained constant up to this weight, or there must have been compensating changes in the contributions to growth from these other sources. Although too few to affect the shape of the curve which has been fitted to the data, the results in the older heavier animals of group 3 suggest that later growth of testicular tissue may come almost entirely from these other sources. Hooker (1944) observed by microscopic examination that the proportion of interstitial tissue in the testes of older animals was much greater than in younger ones.

The curves which relate weight of the testes and age, and mean diameter of the tubules and age, in the Hampshire and Southdown rams of Carmon and Green (1952) are also variable in shape. This variability

may be a reflection, at least in part, of interrelationships between mean cross-sectional area of the tubules, weight of the testes, body weight, and age. However, there do seem to have been some differences between the development of the testes in the Merino rams of the present study and that in the Hampshire and Southdown rams of Carmon and Green (1952). While the testes of the Hampshire rams were of similar weight to those of the Merino rams of corresponding body weight, the testes of the Southdown rams were somewhat heavier. In all except the heavier testes of both the Hampshire and Southdown rams, too, the mean diameter of the seminiferous tubules was smaller than in testes of corresponding weight in the Merino rams; and in the lighter Southdown rams, the seminiferous tubules increased in diameter relative to the weight of the testes at a slower rate than in the heavier Southdown rams.

In relation to body weight, the rate of development of the testes followed much the same course in the animals of all groups. Unless there were compensating differences, therefore, both the amounts of the hormones controlling their growth, and the reactivity of the testes to these hormones, must have been similar in all animals of similar weight. The first obvious change in the morphology of the tubules, vacuolization of the cytoplasm with the formation of a lumen, was observed in all but three instances in animals which weighed from 21 to 28 kg. There is little doubt that gonadotrophic hormones are the main hormones controlling the changes in morphology. At this body weight in most animals of all groups, therefore, either the amount of gonadotrophic hormones, or the reactivity of the testes to gonadotrophic hormones, or both of these factors, had increased sufficiently for obvious differentiation to be initiated within the tubules.

In contrast, relative to that of the testes the rate of growth of the epididymides differed in animals of similar age in groups 3 and 4. Either the amounts of the hormones which control the growth of these organs, or the reactivity of these organs to those hormones, or both of these factors must therefore have varied. The variation is not accounted for by variation in the age or body weight of the animals or the weight of the testes. It must, therefore, have arisen from other differences between the animals of the two groups: the different seasonal conditions, both nutritional and physical, which they experienced during growth in consequence of their being born at different times of the year, and the different rates of growth at the time the epididymides were collected.

That the age at which the prepuce became completely separated from the glans penis was variable has already been noted by Johnstone (1948), Wiggens and Terrill (1953), and Dun (1955). Johnstone's suggestion that the variability was associated with the general rate of growth of the animals is supported by the present observations. There is little doubt that it is a reflection of the relationships between course of separation

and weight of the testes, between weight of the testes and body weight, and between body weight and age. This conclusion is supported by the high correlation which Dun (1955) observed between the state of the penis and the size of the testes.

The rapid increase in the size of the testes as the body weight increased from 23 to 27 kg was accompanied by the appearance of a lumen in the seminiferous tubules, and by progressive separation of the prepuce from the glans penis. With the few exceptions mentioned below, these pubertal changes were confined to animals between 18 and 27 kg in weight.

In almost all of the animals in which spermatozoa were present in the seminiferous tubules of the testes, spermatozoa were also present in the epididymides. In almost all of these animals, too, the prepuce had separated from the glans penis completely. The state of development of the remainder of the reproductive tract is not known, but it is reasonable to assume that these animals had attained puberty or were very close to attaining it. The attainment of this state was associated with both age and general bodily development. It had not been attained in any animal less than 18-20 weeks old. Nor had it been attained by any older animal lighter than 27 kg in weight. On the other hand, it had been attained by all except one of the animals which were older than 20 weeks and heavier than 28 kg in weight. The other older animal, and all younger ones which weighed more than 18 kg, were well advanced towards it. The results of Dun (1955) are in general agreement with these, but one of his rams had certainly attained puberty at a weight lighter than 27 kg.

The potential reproductive capacity of these animals was clearly much greater than that at the attainment of puberty. Much larger testes with larger seminiferous tubules were obtained from animals which were allowed to grow further. Indeed it is doubtful whether full reproductive capacity had been attained by any of the animals, although there is some suggestion that those of group 2 which were 64 weeks old may have done so.

These observations emphasize the importance of plane of nutrition and of good general husbandry in the development of the testes. Since the weight of the testes is related to body weight, the rate of development will be dependent on the rate of growth of the animal. The early attainment of sexual capacity can only be expected when there is a high rate of growth.

One aspect of the early attainment of sexual capacity is the ability of ram lambs to impregnate their dams before they are weaned. It has been reported on a number of occasions that Merino lambs have done so. The present study suggests that it is likely to occur whenever the lambs are well grown, if they are not weaned before they are 5 months old.

V. Acknowledgments

The authors are indebted to the staff of the C.S.I.R.O. Field Stations at Tooradin and Werribee for the care of the animals, to Mr. A. D. Campbell, at that time attached to the Animal Health Research Laboratory, C.S.I.R.O., for the collection of some of the testes, and to Miss H. N. Turner and Dr. E. J. Williams of the Division of Mathematical Statistics, C.S.I.R.O., for assistance in the statistical analysis of the data.

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EFFECTS OF PROGESTERONE AND PREGNANT MARE SERUM (PMS) ADMINISTRATION IN THE ANOESTROUS EWE

I. OESTRUS, OVARIAN CHANGES, AND GONADOTROPHIC POTENCY OF THE PITUITARY GLAND

By J. I. RAESIDE* and D. R. LAMOND†

(*Manuscript received April* 5, 1956)

Summary

Observations were made on the effect of progesterone and pregnant mare serum (PMS) on the incidence of oestrus, the macroscopic changes in the ovaries, and the total content of gonadotrophins in each anterior pituitary gland of 105 anoestrous Romney ewes.

When progesterone alone was administered for 4 days neither oestrus nor ovulation resulted. In the group receiving PMS alone, ovulation occurred in 6 of 10 ewes but none exhibited oestrus. All 10 ewes injected with both hormones ovulated; only 6 came on heat. No effect of progesterone or PMS on pituitary activity was apparent when the animals were killed 4-7 days after the last injection of hormone.

Some evidence for an increase in numbers of small follicles over an 8-day period in January suggested perhaps a natural increase in ovarian activity with the approach of the normal breeding season, or a stimulatory effect of the introduction of the rams at that time of year. Over the same period the gonadotrophic potency of the control sheep showed a slight increase. These observations are discussed in the light of current views of pituitary control of the breeding season in sheep.

I. INTRODUCTION

Within recent years the most successful attempts at synchronization of oestrus and ovulation in the anoestrous ewe by hormone injections have consisted of progesterone administration for several days, with a subsequent injection of pregnant mare serum gonadotrophin (PMS). Even the use of progesterone alone has resulted in oestrus accompanying the induced ovulation in some of the treated ewes (Dauzier and Wintenberger 1952; Dutt 1952, 1953; Robinson 1952, 1954*a*, 1954*b*). A preliminary trial by one of the authors (Raeside, unpublished data, 1954) in November-December suggested that the New Zealand Romney would respond similarly to progesterone alone.

An important factor in such investigations is the time of year at which the treatment is given in relation to the normal breeding season of the sheep. In this connexion information concerning ovarian

* Massey Agricultural College, Palmerston North, New Zealand.

† Massey Agricultural College, Palmerston North, New Zealand; present address: Department of Veterinary Physiology, University of Sydney.

morphology in the anoestrous ewe is limited. Grant (1934), Cole and Miller (1935), and Roux (1936) concluded that the number of visible follicles remains relatively constant throughout anoestrus. Kammlade *et al.* (1952) found that the total number of follicles greater than 1 mm in diameter was significantly less in anoestrus than during the breeding season. There are no comparable data for the New Zealand Romney.

In view of the reciprocal relationships between the anterior pituitary gland gonadotrophins and the ovary, it seemed desirable to make an attempt to assay the pituitary activity in the ewes described in this experiment. Few studies of this nature have been conducted with sheep. Cole and Miller (1935) assayed the pituitary glands of a small number of ewes in different reproductive states and found no notable differences. Both Warwick (1946) and Kammlade *et al.* (1952) compared the total gonadotrophic potency of pituitaries from sheep in anoestrus and in the oestrous cycle. No greater pituitary activity was exhibited during the breeding season; and indeed Kammlade *et al.* (1952) stated that the pituitary potency was uniformly higher during the non-breeding season.

Robinson (1954*b*) reported that while oestrus, presumably with ovulation, was induced in a high proportion of ewes treated with progesterone and PMS in combination, lambing percentages were low.* In an endeavour to discover some of the causes for the low conception rate attending the use of the two hormones, the following experiment was undertaken in early January 1955. This paper presents data on the induction of oestrus, the gross examination of the ovaries, and the gonadotrophic potency of the pituitary glands. An account of the histological examination of the genital tracts will follow later.

II. Materials and Methods

(*a*) *Experimental Animals and Management*

A total of 105 mature Romney Marsh ewes was available for this experiment. All had lambed the previous season in the Massey College flocks but were being culled for age, teeth, or udder troubles.

Four vasectomized and four entire Romney rams were placed with the flock on January 1, 1955, the day before the injections were started. The rams were raddled at least every second day but inspected daily. Observations for oestrus were made twice daily until January 12 and thereafter every morning.

The plan of treatment is outlined in Table 1. Progesterone in arachis oil (12.5 mg/ml) was injected subcutaneously. The subcutaneous administration in aqueous solution of 800 i.u. of a commercial preparation of PMS ("Gestyl") to ewes of group 4 was carried out 50 hr after the

* In a recent trial using artificial insemination it was found that percentage conception and lambing rate were satisfactory in ewes given progesterone and PMS during the breeding season (Robinson 1956).

final injection of progesterone. Injections began on January 2, approximately 2 months prior to the expected commencement of the normal breeding season. Those ewes receiving injections twice daily were segregated with two of the teasers to facilitate handling for the duration of treatment.

TABLE 1

HORMONE TREATMENTS: OESTRUS AND OVULATION

All hormones were administered subcutaneously. The ewes in group 1 and half the ewes in groups 2, 3, 4, and 5 were killed on January 10-13. The remainder were killed on January 18-20

Group	Number of Ewes	Treatments: Progesterone (mg) Jan. 2	Jan. 3	Jan. 4	Jan. 5	PMS (i.u.) Jan. 7	Number Showing Oestrus	Number Ovulating
1	5	100		100				
2*a*	10	50		50				
2*b*	10	25	25	25	25			
2*c*	10	25*	25*	25*	25*			1
3*a*	10	25		25				
3*b*	10	12½	12½	12½	12½			
3*c*	10	12½*	12½*	12½*	12½*			
4*a*	10	25	25	25		800	6	10†
4*b*	10					800		6‡
5*a*	10	1 ml§	1 ml	1 ml	1 ml			
5*b*	10							1

* Given in two equal doses.

† Ovulation was assumed in two ewes which exhibited oestrus but whose ovaries were lost at slaughter.

‡ Ovulation was assumed from histological evidence in the genital tract of one ewe whose ovaries were lost at slaughter.

§ Arachis oil.

On January 10, 11, 12, and 13 the ewes of group 1, as well as half the ewes in each of the other groups, were slaughtered at a local freezing works, care being taken to secure individual identity. The remaining ewes were killed on January 18, 19, and 20, and reproductive tracts recovered. The ovaries were placed in 10 per cent. formalin and examined 2 weeks later, after slicing into sections at 2 mm to allow counting of follicles and examination of corpora lutea.

(*b*) *Assay of Pituitary Gonadotrophin*

The anterior pituitary glands were placed in acetone within 75 min of slaughter, and were stored in acetone for 4 months. They were then dried in a vacuum desiccator, trimmed of obvious extraneous tissue, weighed, and ground. After grinding, the glands were stored in a

desiccator in a refrigerator until they could be assayed in July-August, when chicks became available as assay animals.

The procedure for assaying the pituitaries was similar to the method employed by Kammlade *et al.* (1952). It consisted essentially of suspending 20 mg of the powder in distilled water and injecting the suspension into 2-day-old male chicks. White Leghorn day-old male chicks were obtained from the College Poultry Farm and kept in electric standard chick brooders in a constant temperature room. On the basis of body weight increases in the control birds feeding and management were satisfactory. Injections of 0.2 ml were made twice daily for 4 days, commencing on the day after the chicks were received. Five birds were used for each pituitary and were marked with coloured dyes for identification. Some deaths occurred. Prior to the injections the powder was reground and passed through an 80-mesh sieve. The aqueous suspension of anterior pituitary powder was kept in a refrigerator during the period of the injections.

Twenty pituitaries were assayed individually each week. For comparison between weekly series two levels of PMS (15 and 30 i.u.) were included along with control birds. Chicks were killed using ether on the day after the final injection and weighed immediately after death. On removal the testes were cleaned and blotted before weighing. An increase in weight of the testes of the injected chicks over those of the uninjected controls was taken as a measure of gonadotrophic potency of the sheep pituitary gland.

At the start the pituitaries from groups 2*a* and 3*a* were assayed with 15 mg of powder given intraperitoneally. It was thought that this method might shorten the time taken for injecting, as well as lessen the possible loss of material from the site of injection. However, death losses were heavy, body weights were below normal, and there was no increase in testes weights (Table 3). During the following week the intraperitoneal and subcutaneous routes of administration were compared. The latter method was found to be much more satisfactory. It was also shown at this time that 20 mg of pituitary powder per chick resulted in a greater response, and this level was used in the later assay series.

III. Results

(*a*) *Oestrus*

It can be seen from Table 1 that none of the ewes receiving progesterone alone exhibited oestrus. Failure resulted also from the use of PMS without pretreatment, while 6 of the 10 ewes injected with both hormones came on heat. All of these ewes were served within 2 days of gonadotrophin injection, with the exception of one observed in oestrus on January 11.

(*b*) *Ovulation*

Likewise, it is evident in Table 1 that progesterone injections failed to cause ovulation in the anoestrous Romney ewes. In contrast, PMS alone resulted in 6 of 10 ewes in group 4*b* ovulating. The ovaries were not recovered from one animal in this group, which did not receive progesterone injections, but histological examination of the reproductive tract suggested a recent ovulation. At slaughter, at least one of the ovaries of two ewes in group 4*a* were lost, but since both ewes had been in oestrus it was assumed that ovulation had taken place. Thus when both hormones were given ovulation occurred in all ewes.

TABLE 2

FOLLICLE NUMBERS IN THE OVARIES OF ANOESTROUS EWES

Group	Ewes Killed at First Slaughter			Ewes Killed at Second Slaughter		
	Number of Ewes*	Av. No. of Follicles per Ewe		Number of Ewes	Av. No. of Follicles per Ewe	
		Large (>5 mm)	Small (2-5 mm)		Large (>5 mm)	Small (2-5 mm)
1	4	2.7	8.25			
2*a*	3	2.7	11.33	5	2.4	14.6
2*b*	5	2.5	11.6	4	1.75	18.5
2*c*	4	2.2	14.0	4	3.75	18.5
3*a*	4	1.5	11.5	5	2.0	13.4
3*b*	5	3.4	8.0	5	1.4	21.0
3*c*	3	2.33	12.33	5	2.6	20.3
4*a*	3	2.33	7.0	5	2.2	11.0
4*b*	4	1.0	15.0	4	2.25	23.0
5	9	1.9	14.5	8	1.6	22.2

* Including only ewes in which both ovaries were recovered at slaughter.

The single ovulation in group 2*c* (Table 1) must be considered in the same light as the spontaneous ovulations, unaccompanied by heat, in one of the control ewes.

(*c*) *Ovarian Morphology*

The size and appearance of the induced corpora lutea were similar to those seen in the normal breeding season.

Data on follicle numbers are presented in Table 2, where the average number of ovarian follicles per ewe is given for each group. In considering only follicles 5 mm in diameter or larger, a marked similarity was observed in all groups, the average number being two in each animal. Of the large follicles few were greater than 6 mm and only two ewes had a follicle approaching 10 mm in diameter. Both animals were in group 4 and hence had received PMS.

TABLE 3

ASSAY OF SHEEP PITUITARY GLAND GONADOTROPHIN

The following three ewes were not included in the above table, since each represented the only animal ovulating in its respective group: 57.49 (2*c*(i)), 16.7 ± 0.79 (4 chicks); 50 H (5*b*(ii)), 15.6 ± 0.97 (5 chicks); 229.49 (4*b*(ii)), 22.0 ± 3.07 (5 chicks)

Series	Group	Pituitary Gonadotrophin Content*				15 i.u. PMS		30 i.u. PMS		Control	
		Ewes at 1st Killing		Ewes at 2nd Killing							
		No. of Chicks	Testes Wts. and S.E. (mg)	No. of Chicks	Testes Wts. and S.E. (mg)	No. of Chicks	Testes Wts. and S.E. (mg)	No. of Chicks	Testes Wts. and S.E. (mg)	No. of Chicks	Testes Wts. and S.E. (mg)
1	2*a*	13 (5)†	12.2 ± 1.06	13 (5)	12.6 ± 1.24	4	16.4 ± 2.60	4	22.3 ± 4.1	9	12.6 ± 1.23
	3*a*	16 (5)	11.4 ± 0.99	10 (5)	12.2 ± 1.07						
2	2*b*	25 (5)	16.8 ± 0.90	24 (5)	16.7 ± 0.91	9	21.7 ± 1.13	10	24.9 ± 1.83	10	12.2 ± 0.95
	3*b*	23 (5)	16.4 ± 0.67	22 (5)	16.2 ± 0.81						
3	2*c*	19 (4)	19.4 ± 1.00	25 (5)	19.8 ± 0.93	10	18.9 ± 1.41	10	23.9 ± 2.05	10	13.0 ± 1.35
	3*c*	24 (5)	17.5 ± 0.97	20 (4)	19.3 ± 1.06						
4	5	48 (10)	16.2 ± 0.61	44 (9)	18.9 ± 0.79	9	16.8 ± 0.82	9	28.3 ± 3.09	10	14.7 ± 1.03
5	4*a*	22 (5)	15.8 ± 0.72	24 (5)	16.7 ± 0.77	10	21.3 ± 1.84	10	26.8 ± 2.11	10	13.8 ± 1.36
	4*b*	25 (5)	14.6 ± 0.67	19 (4)	17.6 ± 1.01						

* Estimated by the increase in chick testes weights using five birds for each pituitary gland.

† Numbers in brackets represent number of glands assayed.

Considerable variation was found in the number of small follicles (2-5 mm) per ewe. No significant difference in follicle numbers was encountered between groups at either killing, including the control animals, but there was a significant increase in counts made at the later killings in all groups. This difference was highly significant ($P < 0.01$) in groups 2 and 3, while in the control ewes (group 5) it was significant at the 5 per cent. level ($P < 0.05$). Even in group 4 the difference was evident.

TABLE 4

RESPONSE OF CHICK TESTES TO PMS

PMS Injected per Chick (i.u.)	Number of Chicks	Body Weight (g)	Testes Wts. and S.E. (mg)
10	18	59	15.19 ± 0.72
15	20	60	20.78 ± 1.05
20	20	60	21.89 ± 1.22
30	20	59	24.19 ± 1.49
40	20	64	29.36 ± 1.37
Control	35	60	16.21 ± 1.02

(*d*) *Pituitary Gonadotrophin Content*

The results of the assays of the sheep pituitaries are presented in Table 3. In groups 2, 3, and 4 there was found to be no significant differences in pituitary potency between subgroups and dates of slaughter. However, the differences in activity between dates of slaughter in the control ewes was significant ($P < 0.05$).

TABLE 5

RESPONSE OF CHICK TESTES TO ACETONE-DRIED SHEEP PITUITARY POWDER

Powder Injected per Chick (mg)	Number of Chicks	Body Weight (g)	Testes Wts. and S.E. (mg)
10	11	61	15.06 ± 0.96
15	20	65	17.02 ± 1.24
20	9	65	19.90 ± 1.30
Control	15	61	14.71 ± 0.86

Analyses of the results were performed on logarithmic data to account for differences in variances at different response levels. In Table 4 the increments in testes weights with various levels of PMS are presented. It may be seen that there is an increase in variance with the higher levels of PMS. The logarithmic transformation was found to correct this and the log-dose log-response line ($b = 0.4157$ mg per i.u.) was highly significant. The dose-response relationship between the ewe pituitary material and the immature chick testis is presented in Table 5.

Unfortunately, no comparison between series was possible owing to the degree of variability of the assay animal. A serious problem arose from the variability in response of chicks to the same quantities of PMS in different series (Table 3). Testes weights of the control chicks were somewhat variable and no appreciable advantage was gained from considering the influence of body weight. In addition it was observed that the testes weights of control birds were considerably greater than found by other workers using the same breed. No adequate explanation is available. Bergman and Turner (1942) have drawn attention to the significance of light and temperature as factors affecting testes weights, but since these factors were carefully controlled in this experiment, other possible agents were presumably responsible for the differences between hatches. It may be noted that injections of distilled water alone into 20 chicks did not appear to have any effect on testes weights.

The weights of the acetone-dried pituitary glands ranged from approximately 150 to 400 mg.

IV. Discussion

In this experiment neither oestrus nor ovulation resulted from the administration of progesterone alone. Such a finding contrasts with several reports referred to above. By way of explanation it may be mentioned that both Dutt (1953) and Robinson (1954*b*) have drawn attention to the significance of duration of hormone treatment in relation to response in the ewe. While 1 of 6 Romney ewes came on heat when a total of 150 mg of progesterone was given in five injections over a period of 15 days in a preliminary trial (Raeside, unpublished data, 1954), the plan of the experiment in the later investigation was based on the shorter treatment reported by Robinson (1954*a*) where ovulation was observed in 5 of 6 mature crossbred ewes. Attention therefore is drawn not only to the possible significance of duration of treatment but also to the breed of sheep.

As might be expected, several ewes ovulated without coming on heat when PMS was administered alone. Pretreatment with progesterone resulted in ovulation in the ewes but was not accompanied by oestrus in all animals. It would appear that a greater incidence of oestrus might be achieved by extending the duration of progesterone injections (Robinson 1954*d*, 1955; Wallace 1955). A remarkable difference in lambing results was recorded by these two workers when using the same breed. No doubt much can be attributed to the time of the year at which the hormones were given; Wallace's greater success followed injections in February.

Examination of the condition of the ovaries provides further evidence of the importance of the time of year in such work. Lambourne (1955) has shown an increase in the number of ovulations in late January in the New Zealand Romney ewe. For this reason it seems probable that many of the ewes treated by Wallace (1955) in February had already ovulated.

In the present investigation with similar ewes one control ewe had ovulated and the numbers of small follicles (2-5 mm in diameter) were observed to have increased over an 8 to 10-day period from January 10.

Kammlade *et al.* (1952) found significantly more follicles in ewes during the breeding season than in those in anoestrus. Perhaps then, the approach of the normal breeding season is associated with an increase in numbers of small follicles even preceding the period of "silent" heats. An alternative explanation for the greater ovarian activity in all groups at the second killing may be that the introduction of the rams served as an external stimulus to reproductive activity, as has been reported by several Australian workers (Underwood, Shier, and Davenport 1944; Riches and Watson 1954; Schinckel 1954*a*, 1954*b*). Whatever the underlying cause, our findings emphasize the need for considering such factors in attempting to induce pregnancy in the anoestrous ewe.

Mention has been made above of the work of Kammlade *et al.* (1952), in which it was found that sheep pituitaries contain more gonadotrophic hormone during the anoestrous period than they do on an average during oestrous cycles. Such a finding is at variance with the view generally held that the gonadotrophin content would be expected to be higher during the breeding season (Robinson 1951). It has to be realized that no adequate method for the separate estimation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) is available, so that only total gonadotrophin potency has been measured in this type of study. With this in mind Kammlade *et al.* (1952) postulated that the anoestrous period in sheep is due to an imbalance between FSH and LH, in which there is a rise in FSH activity. Robinson (1954*c*) has questioned this view by noting that the ewe can apparently ovulate all follicles which are developed as a result of the injection of a gonadotrophin, primarily FSH-like in nature.

In considering the results of the present investigation it seems reasonable to relate the slight increase in pituitary activity observed in the control ewes (16.2 mg $\pm$ 0.61 to 18.9 mg $\pm$ 0.79)* to the increase in number of small follicles (14.5 to 22.2 per ewe) over the same period (January 10-18). This finding suggests that the greater potency was due to an increase in the FSH component. It is difficult to reconcile such evidence with the observations of Kammlade *et al.* (1952). No direct comparison is possible, however, since no glands were assayed from ewes in the breeding season in our work.

Several difficulties encountered in the use of the chick assay have been indicated above. A further point which must be considered is the possible loss of potency of the pituitary gland during storage. Naito (1953) reported that acetone-dried goat pituitary glands did not lose potency over a period of 2 years. In total these difficulties present a

* Expressed as chick testes weights.

considerable obstacle in a comparison of the results with the work of other investigators but the present observations point to the need for further studies of this nature to elucidate the factors controlling the anoestrous period in sheep.

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EFFECTS OF PROGESTERONE AND PREGNANT MARE SERUM (PMS) ADMINISTRATION IN THE ANOESTROUS EWE

II. HISTOLOGICAL EXAMINATION OF THE GENITAL TRACT

By J. I. RAESIDE* and D. R. LAMOND†

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Summary

Reproductive tracts from 105 anoestrous Romney ewes were examined histologically. Twenty animals served as controls, 10 received pregnant mare serum (PMS), and 10 were given progesterone injections followed by PMS. Different amounts of progesterone alone were administered during 4 days to the remainder.

Sections were cut from tissues of the vagina, cervix, uterus, and three regions of the fallopian tubes. The findings in the control ewes in the present investigation agreed in general with those reported by earlier workers, with the exception of the fallopian tubes in the anoestrous ewe, for which no data were available in the literature. Apparently the upper part of the genital tract recedes in anoestrus to a condition not observed at any point in the breeding season.

The influence of progesterone injections was most pronounced on the uterus, but was seen to a lesser degree in the cervix. Neither the vagina nor the fallopian tubes exhibited marked changes as a result of progesterone administration but both organs reflected slightly the ovulations which had been induced.

I. INTRODUCTION

No data have been found in the literature concerning the histology of the fallopian tubes in the anoestrous ewe. Cole and Miller (1935) have described the uterus, cervix, and vagina while Grant (1934) studied the vagina and cervix. Both uterus and cervix apparently recede to a state not seen at any period of the normal oestrous cycle. The epithelium lining the uterus and the uterine glands is low columnar and the complexity of the glands is much reduced. An ischemic condition is seen in the endometrial stroma, whose nuclei are shrunken. In the cervix the epithelium and stroma are similarly inactive. However, the vagina does not appear to show the same degree of regression apart from a decrease in height of epithelium. The scant vaginal smear contains fewer leucocytes and epithelial cells.

McKenzie and Terrill (1937) and Bell, Casida, and Darlow (1941) injected castrate ewes with oestradiol benzoate and progesterone. An

* Massey Agricultural College, Palmerston North, New Zealand.

† Massey Agricultural College, Palmerston North, New Zealand; present address: Department of Veterinary Physiology, University of Sydney.

increase in height was observed in the epithelium of the cervix, uterus, and fallopian tubes following oestradiol benzoate injections. The administration of progesterone had no apparent effect on vaginal epithelium but maintained the height of both cervical and uterine glandular and surface epithelia. It was noted also that the injection of progesterone after oestradiol pretreatment resulted in an increase in the coiling of uterine glands.

When ovulations were induced without heat using PMS, Polovceva and Judovic (1939) in a gross examination found the condition of the reproductive tract to be similar to that seen in the breeding season in ewes, except that tonic uterine contractions were absent and there was a tendency for the cervix to remain closed. Bell, Casida, Bohstedt, and Darlow (1941) studied the vaginal smear after induction of ovulation, with or without heat. They used PMS, sheep pituitary extract, and oestradiol benzoate, singly and in combination. The changes noted were similar to those observed during and after natural oestrus, and were independent of whether or not the animal came on heat or ovulated.

The findings reported here are those of an attempt to assess histologically the extent to which the reproductive tract of the anoestrous ewe is restored, by means of progesterone and PMS, to the normal condition of the breeding season.

II. Materials and Method

Reproductive tracts from the ewes treated as described in Table 1 (see also part I of this series) were recovered and labelled at killing when the ewes were sent to a local freezing works. Portions of the fallopian tubes were taken from the fimbriated end, mid region, and utero-tubal junction. Material was obtained from both horns of the uterus, the mid region of the cervix, and the roof of the vagina about 1 in. from the cervix. Tissues were placed in buffered formalin, embedded in paraffin, and cut at 8 μ. For examination, sections were stained with haematoxylin and eosin. Measurements of height of epithelium were taken from three fields, selected at random, in each of four sections in every animal. With recourse to the literature it was possible to make a comparison with the histological appearance of the reproductive organs of the ewe during the breeding season (Grant 1934; Cole and Miller 1935; McKenzie and Terrill 1937).

III. Results

(*a*) *Control Ewes*

The fallopian tubes of the anoestrous ewes were characterized by a decreased size of villi, lower epithelium, and shrunken stromal and epithelial nuclei. In many cases cytoplasmic and nuclear extrusions were common (Plate 1, Fig. 1).

TABLE 1

EFFECT OF HORMONE TREATMENT ON HEIGHT OF UTERINE EPITHELIUM IN ANOESTROUS EWES, 1955

Hormones were administered subcutaneously. Twelve epithelial measurements were made per ewe. The following ewes are not included in the above table because they had ovulated: 57.49 (2*c*(i)), 19.5 ± 3.42 μ; 229.49 (4*b*(ii)), 30.9 ± 3.13 μ; 50 H (5*b*(ii)), 27.1 ± 2.2 μ

Group	Treatments					Ewes Killed at First Slaughter (i)		Ewes Killed at Second Slaughter (ii)	
	Progesterone (mg)				PMS (i.u.)	Number of Measurements	Height of Epithelium and S.D. (μ)	Number of Measurements	Height of Epithelium and S.D. (μ)
	Jan. 2	Jan. 3	Jan. 4	Jan. 5	Jan. 7				
1	100		100			60	21.5 ± 4.17		
2*a*	50		50			60	23.8 ± 4.3	24	14.7 ± 2.65
2*b*	25	25	25	25		60	26.1 ± 4.45	60	17.1 ± 3.46
2*c*	25*	25*	25*	25*		48	22.6 ± 4.57	60	14.0 ± 1.8
3*a*	25		25			60	22.8 ± 3.35	60	14.7 ± 2.17
3*b*	12½	12½	12½	12½		60	22.3 ± 5.07	60	14.5 ± 2.4
3*c*	12½*	12½*	12½*	12½*		60	18.6 ± 2.48	60	14.9 ± 3.71
4*a*	25	25	25	25	800	48	24.9 ± 4.94	60	25.3 ± 3.44
4*b*					800	60	29.6 ± 3.27	48	16.2 ± 1.87
5*a*	1 ml†	1 ml†	1 ml†	1 ml†		48	13.4 ± 2.3	48	14.0 ± 2.15
5*b*						60	13.3 ± 2.45	48	12.3 ± 1.9

* Given in two equal doses.

† Arachis oil given subcutaneously.

Fairly close agreement with reports in the literature was found on examination of uterine sections. The epithelium was almost cuboidal (height 13.2 μ), the crowded nuclei occupying practically the entire cell (Plate 1, Fig. 2); that of the cotyledons appeared to be indistinguishable from the surface and glandular portions. Although the uterine glands were poorly developed, the ducts were numerous and easily seen (Plate 1, Fig. 3). The stroma consisted of densely packed nuclei with occasional large cells containing pigment granules seen in the subepithelial region. Numerous lymphocytes were present, being most frequent near the basal layer of the glandular and surface epithelium. The uterine glands were seen to be shrunken and their basal nuclei were small, elongated, and crowded. The numerous blood vessels contained few red blood cells.

Pseudo-stratified columnar epithelium lined the cervical folds and was 18-20 μ in height. Both epithelial and stromal nuclei were shrunken. Activity at the base of the branches of the cervical glands was less than that seen in the normal breeding season, although some cells were distended, presumably with mucus.

The general picture in the vagina was as follows. It was most common to find the epithelium composed of fairly regular cell layers, the outer one of which consisted of polyhedral cells with well-defined nuclei (Plate 1, Fig. 4). The height of the epithelium averaged 65 μ with a range of 25-190 μ. While the epithelial cell membranes were generally clearly defined and the cytoplasm mainly globular, there was some evidence of vacuolar degeneration in all cases. The basal nuclei were predominantly plump and clear. In some cases there was a slight amount of cornification but desquamation was not obvious. In each field the number of leucocytes varied from about 40 to 120.

From the above description it was seen that the upper part of the reproductive tract had receded in anoestrus to a state not observed at any point in the breeding season. On the other hand the vagina was not unlike that recorded for ewes in pro-oestrus.

(*b*) *Ewes Injected with Progesterone*

Treatments involving progesterone alone were revealed only in those ewes slaughtered at the earlier dates. At the second killing the tract had apparently receded to the normal anoestrous state. The most striking changes occurred in the uterus. There were also changes in the cervix and the isthmus, or uterine end of the fallopian tube. The upper part of the fallopian tubes and the vagina did not show any obvious signs of alteration.

All levels of progesterone caused more or less identical changes in the uterus (Plate 1, Figs. 5 and 6). They consisted mainly of an increase in height of epithelium (see Table 1), an increase in coiling of the

uterine glands, and increased vascularity and oedema of the stroma. The epithelium of the isthmus also increased in height.

The changes in the cervix were most noticeable with the higher levels of progesterone (groups 1, 2*b*, and 2*c*). At the lower levels there was evidence of increased vascularity and oedema, while at the higher levels the epithelium also showed a response by an increase in height, taking on a simple, tall columnar appearance.

(*c*) *Ewes Receiving PMS Alone and Following Progesterone*

In group 4*a* at both dates of slaughter the uterine sections showed greater development of glands and folding of the surface epithelium (Plate 1, Figs. 7 and 8). All ewes in this group had ovulated. In group 4*b*, in which only six had ovulated and in which there had been no priming with progesterone, the uterus of those which had ovulated was similar at the first killing to those of group 4*a*. However, at the second killing they were similar to those of groups 1, 2, and 3. The ewes which had not ovulated in group 4*b* were indistinguishable from the controls. Thus the response in the uterus to injections of progesterone had been enhanced by ovulation (see Plate 1, Figs. 5 and 7).

Similarly in the cervix there was greater development of epithelium and more branching at the base of the glands.

The sections of the upper portions of the fallopian tubes indicated that ovulation in the anoestrous ewe had been associated with an increase in vascularity and oedema of the mucosal folds. Also, some development of the epithelium was noted by an increase in height and a plump appearance of the nuclei. These changes, however, were not marked.

In the vagina there was evidence of cornification in the ewes of group 4*a* which were killed a few days after ovulation (Plate 1, Fig. 9). Cornification was not so easily seen in the ewes in group 4*b* which had ovulated a few days prior to slaughter. Other changes in the vagina in these groups were not obvious.

IV. Discussion

The problem of out-of-season breeding of ewes appears to be entering another phase. Synchronization of oestrus and ovulation may be expected in a high proportion of sheep treated with progesterone prior to an injection of PMS. Although much work is still required to establish a practical basis for the application of such knowledge, the emphasis is shifting from ovulation and oestrus to conception rate and lambing results. It becomes of some significance to ask to what extent the reproductive tract of the anoestrous ewe has been stimulated by these hormones.

The results presented in this paper show that the uterus was very responsive to the progesterone administration; presumably it would have

been sufficiently receptive to a fertilized egg. Similarly the cervix, while requiring more progesterone than the uterus, did respond markedly. On the other hand there is considerable doubt whether the fallopian tubes revealed any signs of stimulation from progesterone. McKenzie and Terrill (1937) demonstrated the effect exerted by oestrogens on the histology of the fallopian tubes and suggested a significance for it in egg and sperm transport through the tube.

A comparison of groups 2*b* and 4*a* indicates the importance of oestrogen, in this instance from the follicles before ovulation, in a full progestational response in the endometrium (Plate 1, Figs. 3, 5, and 7). Again in the cervix, the presence of an ovulation has considerably enhanced the effect of progesterone alone. Further work would seem desirable to establish quantitatively these relationships in the ewe, as has been done for the oestrus response by Robinson (1955), who also reported that progesterone pretreatment increased the reactivity of the vaginal epithelium to oestrogen. In this connexion the histological finding of greater vaginal cornification in group 4*a* than in 4*b*, in ewes which were killed a few days after ovulation, seems to add support to Robinson's evidence for the importance of progesterone stimulation *before* oestrogen in certain reproductive phenomena of the ewe.

No apparent advantage from the prior treatment with progesterone was detectable in changes in the upper portions of the fallopian tubes subsequent to ovulation. Thus the findings of this experiment are in general agreement with the view that in the ewe the influence of progesterone on the histology of the genital tract is most pronounced in the uterus, and to a lesser extent in the cervix. Both the vagina and fallopian tubes are more susceptible to oestrogenic stimulation.

V. Acknowledgments

The authors wish to thank Mr. L. B. Anderson and Mr. H. J. Drake, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North, for the photographic work.

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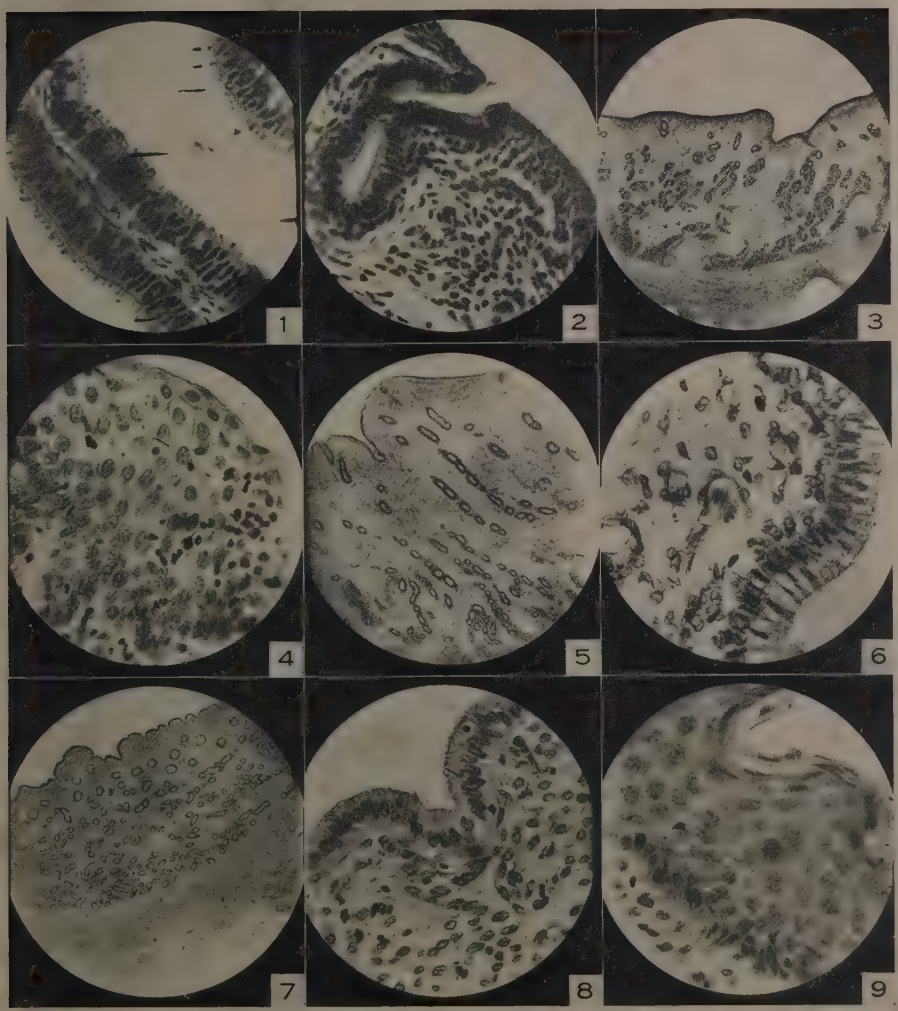
PROGESTERONE AND PMS IN THE ANOESTROUS EWE. II



Explanation of Plate 1

Fig. 1.—Photomicrograph of mid region of fallopian tube of a ewe in anoestrus. × 260.

Fig. 2.—Photomicrograph of intercotyledonary uterine epithelium of a ewe in anoestrus. × 260.

Fig. 3.—Low-power photograph of intercotyledonary endometrium of a ewe in anoestrus. × 26.

Fig. 4.—Photomicrograph of vaginal epithelium of an anoestrous ewe. × 260.

Fig. 5.—Low-power photograph of intercotyledonary endometrium of a ewe which received progesterone alone. × 26.

Fig. 6.—Photomicrograph of intercotyledonary epithelium of a ewe which received progesterone alone. × 260.

Fig. 7.—Low-power photograph of intercotyledonary endometrium of a ewe which received progesterone and PMS. × 26.

Fig. 8.—Photomicrograph of intercotyledonary epithelium of a ewe which received progesterone and PMS. × 260.

Fig. 9.—Photomicrograph of vaginal epithelium of a ewe which received progesterone and PMS. Note cornification of superficial cells. × 260.

THE EFFECTS OF SOME CHLORINATED HYDROCARBONS AS SYSTEMIC ACARICIDES AGAINST THE CATTLE TICK, *BOOPHILUS MICROPLUS* (CANESTRINI)

By W. J. ROULSTON*

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Summary

Subcutaneous injections of cattle with lindane, dieldrin, and aldrin solutions in peanut oil at the rate of 25 mg toxicant/kg body weight produced marked effects on infestations of *Boophilus microplus* (Canestrini). The numbers of ticks present at the time of the injection declined, but many developed to maturity. The most striking effect was the prevention of establishment of seed ticks. After injection, the average period of freedom from larvae for the lindane- and dieldrin-treated animals was from the 7th to the 22nd day and from the 12th to the 25th day respectively. For the aldrin-treated group there was a minimum number of larvae from the 16th to the 27th day.

Dieldrin and aldrin continued to affect the reinfesting ticks even after they were able to establish as larvae, and the first appearance of each succeeding life history stage was progressively postponed. Animals injected with lindane exhibited their first persistent reinfestation of young adult ticks on the average 43 days after injection, dieldrin-injected animals 67 days after injection, and aldrin-injected animals more than 80 days after injection.

Lindane was detected in the hair of injected cattle in three out of four experiments, but could not be detected in skin and blood samples.

DDT injections at 25 mg/kg were without effect, and DDT could not be detected in skin, hair, and blood samples.

Toxaphene, diazinon, and malathion produced no systemic effects on ticks when injected subcutaneously into cattle at 25 mg/kg. Isodrin likewise produced no systemic effect on ticks when injected into cattle at the rates of 5, 10, and 15 mg/kg. Endrin at 5 mg/kg was also ineffective, but two animals injected respectively with 10 and 15 mg/kg endrin died. Compound L13/59, an organic phosphorus acaricide, produced no effect on ticks when administered orally to cattle at rates up to 50 mg/kg.

Inunction of the neck of cattle with peanut oil solutions of lindane and dieldrin at the rate of 25 mg toxicant/kg body weight resulted in marked mortality of ticks over the entire body region, suggesting penetration of the skin and systemic effect from these toxicants. The lindane-anointed animal was free from larval ticks from the 3rd to the 11th day after treatment, and the dieldrin-anointed animal from the 3rd to the 21st day. Similar treatments with aldrin and DDT at 25 mg/kg were without systemic effect on the ticks.

Although these results of injection are promising, the technique cannot yet be recommended for general use.

* Division of Entomology, C.S.I.R.O., Veterinary Parasitology Laboratory, Yeerongpilly, Qld.

I. Introduction

Parasitologists have long been attracted by the prospect of introducing into the bodies of vertebrates chemicals which would destroy ectoparasites without harming the host. Cattle have played an important part in such investigations, as the economic importance of their parasites warrants. As early as 1892 Francis reported negative results from feeding sulphur and salt to a tick-infested bull for a fortnight. Cooper and Laws (1915) injected arsenic subcutaneously into cattle, but found that only very local effects were produced on the tick population, while oral administration was valueless, and topical application of concentrated solutions did not affect the general tick population. Bruce (1940) demonstrated that intravenous injection of arsenic was ineffective against horn-flies attacking cattle. Knipling (1938) found that oral administration of phenothiazine to cattle was ineffective against screw-worm larvae. Spence (1952*a*) found that fortnightly administration of phenothiazine to cattle produced no effect on infestations of *Hypoderma* species, but Schwartz, Porter, and Herlich (1954) allowed cattle free choice of phenothiazine in a mineral mixture, and demonstrated a significant reduction in the numbers of cattle grubs reaching their backs.

Organic insecticides ultimately provided an array of chemicals with properties previously unknown amongst inorganic insecticides. Wilson (1948) showed that oral administration of BHC to a calf for four successive days made it toxic to tsetse flies and ticks for the succeeding 37 days. Barlow (1947) demonstrated by chemical and biological tests that BHC persisted in the blood of cattle for some time after oral administration. Mocsy (1947) reported the control of lice on cattle by the oral administration of certain contact insecticides. Lindquist *et al.* (1953) found that subcutaneous injections of certain insecticides made calves toxic to deer flies and mosquitoes. The United States Bureau of Entomology (1953) reported successful control of cattle grubs and screw-worms by subcutaneous injection of lindane, dieldrin, and aldrin. McGregor, Radeleff, and Bushland (1954) found that subcutaneous injection of diazinon, compound L13/59, and chlorthion killed high percentages of cattle grubs present in the backs of cattle at the time of treatment. Eddy *et al.* (1954) found that cattle remained toxic to horn-flies for 21 days after oral dosage of lindane ceased. McGregor *et al.* (1955) demonstrated that subcutaneous injections of lindane, dieldrin, and aldrin into cattle controlled screw-worms and cattle grubs.

It is apparent from the work reviewed above that the investigation of systemic toxicants against cattle pests is thoroughly justified, and this is confirmed by other papers on systemic effects of chemicals on ecto-parasites of hens, rabbits, mice, and pigs. Since the advent of organic acaricides, no attention has been paid to systemic effects against *Boophilus* species, which would be expected to be more amenable to control by this

method than blood-sucking flies or three-host ticks, because of the prolonged and intimate contact of each individual tick with the one host during its entire parasitic life history. From this aspect *Boophilus* species afford a closer parallel to lice and cattle grubs. Experiments were therefore carried out with substances which had shown promise in the work of previous investigators. The method of oral administration was rejected because of the possibility of direct transfer of acaricides to parts of the skin during licking. The limited work published on intravenous injection did not encourage the use of this method. Therefore subcutaneous injection of the acaricides was adopted, supplemented by inunction experiments.

II. Methods

The dosage of 25 mg/kg body weight was selected, since the United States Bureau of Entomology (1953) reported no ill effects in cattle following injection with lindane, dieldrin, and aldrin at this rate.

Weighed quantities of lindane, *pp'*-DDT, dieldrin (recrystallized from acetone), and aldrin (recrystallized from methanol) were dissolved in the minimum quantities of peanut oil. Half the required amount was injected on each side of the body under the loose skin behind the fore leg. The injection sites were clipped and swabbed with alcohol before treatment.

In the inunction experiment the required amounts of solution were rubbed into the neck close behind the head, where the animal could not lick.

The trials were carried out at the Amberley Field Station, C.S.I.R.O., in south Queensland, using grade Shorthorn cows varying in weight, as calculated from their dimensions (Anon. 1954), from 500 to 900 lb.

Each of the animals was examined carefully at intervals of a few days throughout the experiments, and the infestation of the 12 parasitic life cycle stages recognized by Hitchcock (1955) recorded separately as nil, very light, light, moderate, heavy, or very heavy, or 0-5 respectively when numerical data were needed for graphing. Attempts to develop a truly quantitative method of tick population assessment have so far proved unsuccessful or so time-consuming as to limit observations severely.

The first two experiments were carried out during a period of seasonal scarcity of seed ticks in the pastures. Some doubt would necessarily exist, therefore, whether failure of the animals to become reinfested was due to the low seed tick numbers or to the continuing effect of acaricides. When examinations of untreated animals suggested low pasture populations of seed ticks, large cultures of healthy seed ticks were applied to each animal at intervals. Massive applications of seed ticks were also made to the grass in the vicinity of water troughs in the paddock where the animals were kept between observations. In the interpretation of results, however, reliance is placed principally on the

most comprehensive injection experiment (3), which was carried out during the time of natural tick abundance, and without recourse to artificial infestation.

Hair, blood, and skin samples were taken from the animals injected with DDT and lindane and, for comparison, corresponding samples were taken from untreated animals and animals injected with peanut oil alone. DDT analyses were carried out by the Downing and Norton (1951) modification of the Schechter-Haller method, and lindane analyses by the method of Schechter and Hornstein (1952). No suitable microchemical methods were available for the detection of dieldrin and aldrin.

III. Results

(*a*) *Subcutaneous Lindane, Dieldrin, and Aldrin*

(i) *Autumn* 1954 (*Experiment* 1).—Three animals moderately infested with all stages of ticks were injected with lindane, dieldrin, and aldrin respectively. At 2 and 5 days after injection, all stages of ticks were still present, apparently unaffected, and as there seemed no need for further studies, the lindane-injected animal was re-treated 7 days after the initial treatment to see whether repeated dosage would produce any result, a move which proved to be premature. No larvae were found on the lindane-injected beast from the 7th day after the initial injection. On the 12th day after injection this animal and the one injected with dieldrin were free from both larval and nymphal ticks, whilst that injected with aldrin was devoid of larval ticks. The treated animals gradually lost the ticks present at the time of injection as these matured and fell, and by the 34th day after injection no survivors remained. No reinfesting larval ticks were found until the 27th day after treatment, when a few appeared on the aldrin-injected animal. Live larval ticks were not found on the lindane- and dieldrin-injected animals until the 34th day after treatment. Subsequent examinations, however, showed that these larval ticks failed to survive.

The natural tick infestation on untreated animals had been diminishing since the commencement of the experiment, and artificial infestation, as previously described, was therefore resorted to intermittently. Study of untreated animals running in the same paddock indicated that the scattering of larvae near the water troughs ensured reinfestation. On the 48th day after treatment, about 40,000 larval ticks were placed on the backs of each of the three treated animals. A noticeably smaller number of ticks reached each successive life history stage after the establishment of the larvae. When the animals were stalled to recover the engorging female ticks, less than 20 were collected from each. Mortality of immature ticks at various stages has been reported even on untreated animals (Hitchcock 1955), but reductions of the above order suggest that the toxic effect of the injections was still operating, though with reduced intensity. Not until about the 100th day were adult ticks produced by

TABLE 1

THE EFFECTS OF SUBCUTANEOUS INJECTIONS OF LINDANE, DIELDRIN, AND ALDRIN ON SUBSEQUENT INFESTATIONS OF *BOOPHILUS MICROPLUS* ON CATTLE

Animals examined at intervals as in Figs. 1 and 2 (experiment 2) and Figs. 3-5 (experiment 3)

Toxicant	Animal Number	First Examination Day After the Injection to Observe: Absence of All Stages of Larvae	Reappearance of Larvae	Continuous* Reinfestation of Developing Larvae	Continuous* Reinfestation of Engorged Larvae	Reappearance of Young Adults	Continuous* Reinfestation of Young Adults
(*a*) *Experiment* 2: *injected* 21.vii.1954							
Lindane	1	9	30	37	41	55	55
	2	6	27	27	41	48	48
Dieldrin	3	13	16	27	51	72	128
	4	13	30	30	72	110	110
Aldrin	5	20	30	30	55	92	92
	6	20	27	27	27	65	65
(*b*) *Experiment* 3: *injected* 12.x.1954							
Lindane	7	7	14	21	27	48	48
	8	7	27	27	30	48	48
	9	7	27	27	30	41	48
	10	7	27	27	30	37	37
	11	7	14	14	27	37	37
	12	7	14	27	34	27	41
Dieldrin	13	9	27	34	37	48	48
	14	9,† 14,† 16,† 21†	27	27	51	73	73
	15	9	21	21	30	41	58
	16	14	30	30	44	44	58
	17	14	27	27	48	62	62
	18	14,† 16,† 21†	27	27	51	66	66
Aldrin	19	14	30	30	62	91	91
	20	16,† 21,† 27†	‡	30	37	48	62
	21	16,† 21,† 27	30	30	58	91	91
	22	14,† 16,† 21,† 27†	30	30	62	66	>104
	23	14	16	27	80	91	91
	24	27§	‡	27	37	34	48

* "Continuous", stage detected on two successive examination days.
† Except for young larvae which apparently did not persist.
‡ No break in infestation.
§ Minimum infestation: animal believed to have received less than intended dosage.

the injected animals from larvae picked up from the cultures scattered around the watering points.

(ii) *Winter* 1954 (*Experiment* 2).—Pairs of animals were injected subcutaneously with peanut oil solutions of DDT, lindane, dieldrin, and aldrin at the rate of 25 mg/kg. The eight injected together with four untreated animals were allowed to run in a paddock, which was kept artificially infested with larval ticks. Before treatment the animals carried light to moderate infestations of all stages of ticks but, when the numbers on the control animals declined, even though the natural infestation on the pasture was artificially supplemented, recourse was had to applying larvae directly to the animals. In order to avoid tick worry on the control animals, they received considerably smaller doses than the groups which had been injected.

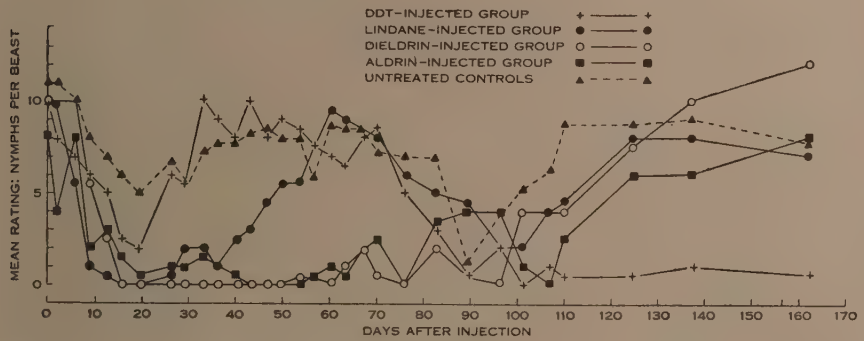

Fig. 1.—Experiment 2: mean ratings of *Boophilus microplus* nymphs per beast on a pair of untreated bovines and on pairs of bovines injected on day 0 with 25 mg/kg DDT, lindane, dieldrin, or aldrin.

After varying periods, all lindane-, dieldrin-, and aldrin-injected animals became free from larval ticks, and remained so for a time (Table 1(*a*)); but the DDT-injected and the untreated animals carried larval ticks continuously.

Pronounced reductions also occurred in the numbers of nymphs on the lindane, dieldrin, and aldrin groups after injection. Increases in the numbers of this instar commenced on these animals after the 36th, 51st, and 55th days respectively, though the DDT-injected and untreated animals carried considerable numbers of nymphs throughout (Fig. 1).

The lindane-injected animals were free from adult ticks from the 34th to the 44th day, the dieldrin-injected animals from the 27th to the 69th day, and the aldrin-injected animals from the 34th to the 62nd day. The subsequent history of the young adult infestation on these three groups is shown in Table 1(*a*). The DDT-injected and untreated animals carried adult ticks of all stages throughout.

The ratings of the mean tick populations on the animals in each treatment are compared in Figure 2. The infestation on the control animals in this figure might be taken to indicate that the depression in the tick population on injected animals was largely due to seasonal effects on seed tick populations in the pastures; but had the control animals received artificial infestation equal to that applied to the injected animals, a much greater contrast would undoubtedly have resulted. Although DDT

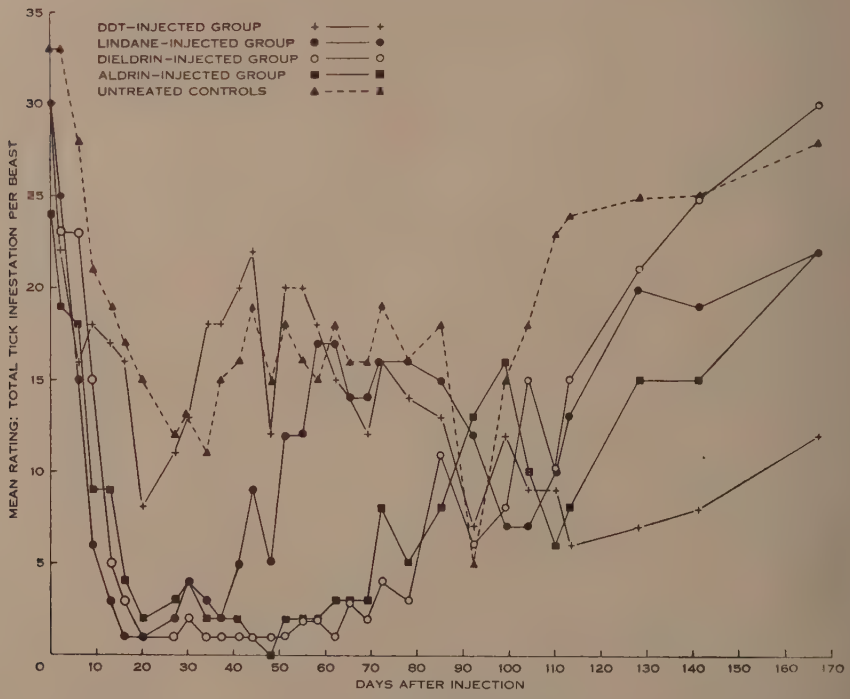

Fig. 2.—Experiment 2: mean ratings of total *Boophilus microplus* infestation per beast on a pair of untreated bovines and on pairs of bovines injected on day 0 with 25 mg/kg DDT, lindane, dieldrin, or aldrin.

injections did not produce any effect on the ticks during the first few months, the isolated examinations made from the 113th to the 167th day indicated that the infestations on these animals were consistently much lighter than on the other groups, particularly the controls and the animals injected with dieldrin and lindane, whereas all infestations at the commencement of the experiment were closely comparable. More data are needed, however, before this could be attributed to an effect from DDT.

(iii) *Spring and Summer* 1954-1955 (*Experiment* 3).—In the spring of 1954, when there were indications of a seasonal increase in the natural

tick population at Amberley, groups of six cows each were injected subcutaneously with DDT, lindane, dieldrin, and aldrin respectively at the rate of 25 mg/kg body weight. Six untreated cows were retained as controls. Tick infestations were light to moderate at the start of the experiment, throughout which all animals were maintained in one paddock.

The effects of the injections on the ticks were generally similar to those observed in the two earlier experiments. There was a noticeable reduction in the number of ticks present at the time of injection, though many were able to develop to maturity. The summary of observations on

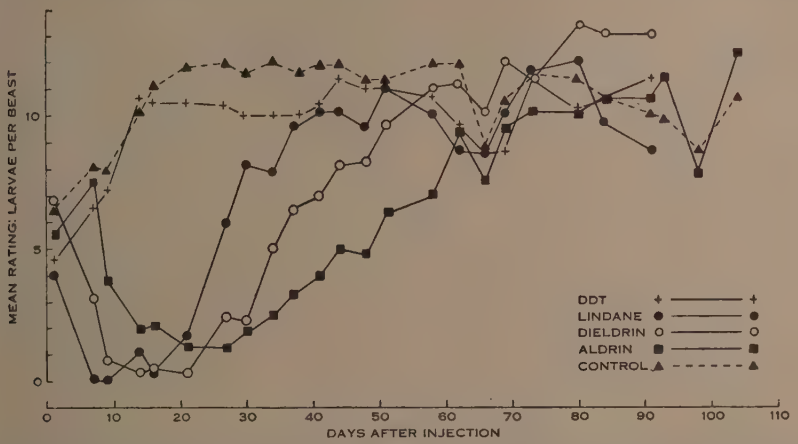

Fig. 3.—Experiment 3: mean ratings of *Boophilus microplus* larvae on six untreated bovines and groups of six bovines injected on day 0 with 25 mg/kg DDT, lindane, dieldrin, or aldrin.

reinfestation presented in Table 1(*b*) needs little qualification. After the fall of the original infestation, the first engorged adults appeared on one animal of the lindane-injected group on the 44th day, which was 23 days after the larvae began to attach in large numbers. The first engorged adults from larvae attaching after dieldrin injection were recorded on one animal on the 58th day, 31 days after the larvae had begun to attach. All other animals of this group produced their first engorged adults even later.

The aldrin-injected animals did not become entirely free from larval ticks, but carried the lowest numbers on the 21st day, when only newly attached larvae were present, except on one cow which was known to have received the complete amount of the injection on one side only, because of a fault in the syringe. Throughout the experiment, this animal exhibited far less reduction in tick infestation than the other animals in the group. There was no sharp increase in the numbers of larvae attaching to the aldrin-injected animals, such as occurred on the lindane-injected

animals between the 14th and 27th days and on the dieldrin-injected animals between the 21st and 30th days; but a slow build-up took place from about the 27th day. The first engorged adults were recorded on one

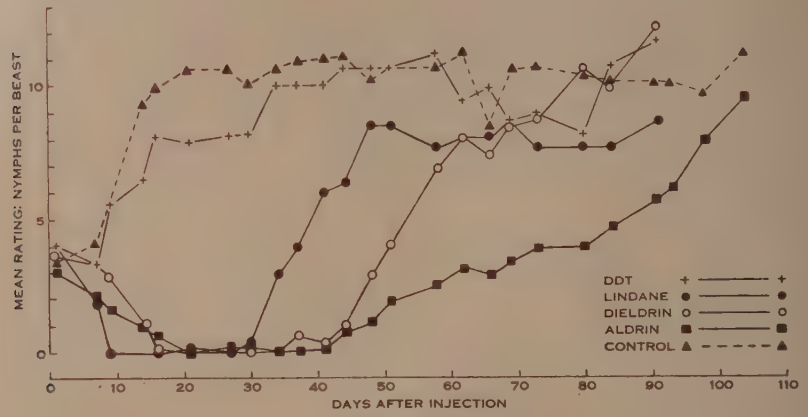

Fig. 4.—Experiment 3: mean ratings of *Boophilus microplus* nymphs per beast on six untreated bovines and on groups of six bovines injected on day 0 with 25 mg/kg DDT, lindane, dieldrin, or aldrin.

aldrin-injected animal on the 66th day. This was 39 days after the larvae had started to attach in large numbers.

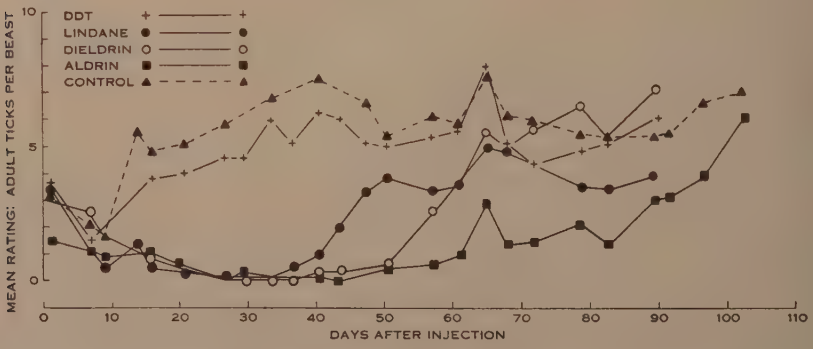

Fig. 5.—Experiment 3: mean ratings of adult *Boophilus microplus* per beast on six untreated bovines and groups of six bovines injected on day 0 with 25 mg/kg DDT, lindane, dieldrin, or aldrin.

The six untreated animals and the six animals injected with DDT continually became infested with fresh larval ticks, and each animal carried an infestation of all stages of ticks throughout the experiment. Graphs of the numerical ratings of the larval, nymphal, and adult stages on the five groups are given in Figures 3-5.

The mean parasitic life cycle of female *B. microplus* is 22 days (Hitchcock 1955). The mean periods elapsing between the attachment of larval ticks, after the maximum effect of the acaricidal treatments had passed, and the appearance of engorged females, were 23, 31, and 39 days for the lindane, dieldrin, and aldrin groups respectively. This suggests that, after the completely prohibitive toxic effects of the injections were past, the first ticks attaching to the animals of the lindane group were able to develop normally to maturity, whereas those which first attached to the dieldrin or aldrin groups either died or had a greatly extended life cycle. It is believed, from the detailed observations made on the cattle, that the ticks died at various stages of development, each succeeding wave of attaching larval ticks developing to a more advanced stage, until finally the ticks were able to develop normally to fully engorged adults.

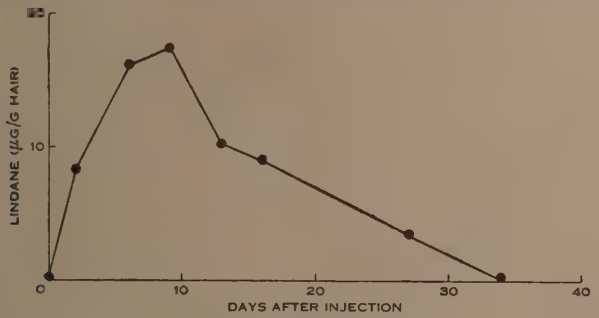

Fig. 6.—Experiment 2: amounts of lindane found in hair of cattle at intervals after injection on day 0 with 25 mg/kg lindane.

(*b*) *Experiments with Other Toxicants*

Tests were made to determine if other toxicants, effective as contact acaricides, had any systemic effects. Toxaphene, diazinon, and malathion were injected subcutaneously into cattle in peanut oil solution at the rate of 25 mg/kg body weight, and isodrin and endrin at the rates of 5, 10, and 15 mg/kg. None of the injections had any apparent effect on the ticks. The two animals injected with the higher dosages of endrin died before any systemic effects on the tick infestation were observed.

Compound L13/59,* an organic phosphorus acaricide, could not be dissolved in peanut oil, and was administered orally in gelatine capsules at the rates of 25, 37.5, and 50 mg/kg. It too was without effect on the ticks.

(*c*) *Chemical Studies of Lindane and DDT*

In experiment 1, hair samples taken from the animal injected twice with lindane were found to contain lindane up to the 48th day after the

* Farbenfabriken Bayer, Leverkusen, Germany.

initial injection. Lindane was also detected in the hair taken from each side of the two animals injected with lindane in experiment 2 (Fig. 6); but no DDT could be found in the hair samples taken from either animal.

Hair, skin, and blood samples were taken from two of the animals in both the lindane and DDT groups in experiment 3 before treatment, and further blood samples taken 2 hr after the injection. Hair, skin, and blood samples were also collected 1, 3, 7, 10, and 14 days after injection. No lindane or DDT was detected in any of the samples, even the hair, a result in contrast with the earlier experiments. At the time of commencing experiment 3 the animals were noticeably shedding their winter coats, but it is not known to what extent this may have affected the results. Presumably, however, the newly growing coat would be so short as to be poorly represented in the samples.

The conflicting results of hair samples taken from animals injected subcutaneously with lindane led to the commencement of experiment 4 on December 6, 1954. Two animals were injected with peanut oil solutions of lindane at the rate of 25 mg/kg body weight, one animal was injected with a similar quantity of peanut oil alone, and a fourth animal was retained as an untreated control. Hair, skin, and blood samples were taken at intervals similar to those in experiment 3 and analysed for lindane. Separate analyses for lindane were made on the hair samples from each side of each animal. The hair from the lindane-injected animals gave a mean value of 17 μg lindane per g hair on the 3rd day after the injection, but no lindane was detected on the 7th or any succeeding day. No lindane was detected in skin samples equivalent to an area of 4 sq. cm per animal. It was not possible to obtain any conclusive evidence for the presence of lindane in 100 ml of blood because of high blank values.

(*d*) *Effects of Topical Application of Toxicant Solutions*

As acaricidal effects over the whole body resulted from purely local subcutaneous injections, it appeared possible that a similar effect might result from the application of small quantities of concentrated solutions to a limited skin area of the animal.

In June 1954, four moderately tick-infested animals were treated respectively with peanut oil solutions of DDT, lindane, dieldrin, and aldrin. The solutions were applied at the rate of 25 mg acaricide/kg body weight to the neck (where the animals were unable to lick) and rubbed well into the hair.

Three days after treatment no young or medium-sized larvae could be found on the dieldrin-treated animal, even on the posterior areas of the body. Seven days after treatment the youngest ticks surviving were fully engorged nymphs, but many of this stage were also dead. The animal was free from larval and nymphal ticks when examined on the 13th and 17th

days after treatment, but by the 21st day newly attached and medium-sized larvae were found, suggesting that the treatment had protected the animal against larval infestation for about 18 days.

The lindane-treated animal was free from larval ticks on the 3rd day and from larvae and young nymphs on the 8th day. All larval stages were present again on the 13th day, suggesting that lindane inunction on the neck had afforded a protective period of 8 days against the attachment of larvae over the whole body.

The aldrin- and DDT-treated animals were apparently not protected against the attachment of larvae.

Control animals running in the same pasture continually picked up small numbers of larvae from the cultures scattered in the vicinity of the water trough.

IV. Discussion

The experiments showed clearly that subcutaneous injections of cattle with peanut oil solutions of lindane, dieldrin, and aldrin produced profound effects on cattle tick infestations. At the rate of 25 mg of toxicant/kg body weight, the destruction of ticks present at the time of injection varied with the toxicant, but for the most part was incomplete, the really significant effect being prevention of larval establishment.

Lindane affected the ticks most rapidly, but was not as persistent as the other acaricides. On the other hand, aldrin was slowest to reduce the tick infestation, took longest to kill invading larvae (Table 1), and exerted the most persistent effects on the parasitic population. These differences in rapidity of action are illustrated in the protective periods* as determined from the various life history stages of the tick. In the most comprehensive experiment (3) the mean protective period for the six animals injected with lindane, based on the earliest reappearance of persistent young larvae, was 16.8 days. For dieldrin, and particularly for aldrin, a clear-cut period of protection based on the earliest reappearance of young larvae could not be determined, because the slower effect of the toxicant on invading larvae permitted some development before death and resulted in an overlap of the populations. The respective mean protective periods for lindane, dieldrin, and aldrin, obtained by subtracting from the mean time of first reappearance of persistent engorged larvae the normal time required to attain this stage (3.7 days), were 18.7, 28.0, and 35.2 days, whilst the mean protective periods based

* "Protective period" as used in this discussion is not identical with the term as used by Norris, Roulston, and Snowball (1950) in dipping trials where the protective period commences immediately after treatment. In these trials the protective period is calculated from the first examination date that the animals are either free from, or carrying a minimum number of, larvae, which in practice means that the protective period commences at least a week after treatment.

on the times of reappearance of young adult ticks (stage attained on average 14 days after attachment of larvae) were 22.2, 35.3, and > 50.3 days (i.e. 3.5, 7.3, and > 15.1 days longer than the corresponding periods based on engorged larvae). The consistently longer periods obtained by basing the protective periods on young adults rather than on engorged larvae, and the progressive increase in the differences between the figures obtained with the later stage throughout the series lindane: dieldrin: aldrin, illustrate the differences in rapidity of toxic effect and persistency alluded to above.

The dosage of 25 mg/kg body weight used in the present experiments is probably close to the minimum necessary to produce an acaricidal effect with dieldrin. Evidence that this might be so was obtained by a central Queensland grazier who, subsequent to the performance of the first C.S.I.R.O. systemic experiments, injected a herd of cattle of various ages uniformly with sufficient peanut oil solution of dieldrin for a 533 lb beast at 25 mg/kg. In this case it was reported that only the smaller animals became free of ticks, so that presumably only these received sufficient dieldrin. Indications that the dosage of 25 mg/kg might also be near the critical lower limit of aldrin were obtained in experiment 3, in which animal 24 received less than the full dose and showed a markedly inferior kill of ticks and less persistent effects (Table 1).

Possibly the dosage of 25 mg/kg may also be near the critical lower limit for lindane, but there is no direct evidence relating to cattle ticks. Lindquist *et al.* (1953) found rather critical lower limits of dosage at which lindane would kill mosquitoes by systemic action, but it is doubtful if their results can be applied logically to cattle ticks.

The mode of action of systemic acaricides is not understood. Menon, Sen Gupta, and Basu (1951) found that oral administration of insecticides to chickens caused a high mortality of blood-feeding ticks, but lice, feeding only on detritus of epidermal structures, were also killed. Most workers on the systemic effect of toxicants, however, appear to have assumed that the ectoparasites were killed exclusively by imbibing blood containing a lethal concentration of insecticide. Barlow (1947) showed that BHC persisted in the blood of cattle up to 29 days after four daily oral doses at the rate of 250 mg/kg. In the experiments described in the present paper, analyses of blood samples taken after the subcutaneous injection of single doses of lindane or DDT at the rate of 25 mg/kg were negative. The possibility that minute traces of the toxicant were present (method sensitive to 10 μg lindane in 5 ml blood) cannot be dismissed, but on the whole, the evidence is against the blood-stream being the direct source of the toxicant destroying the cattle ticks. Eddy *et al.* (1954) found that the blood from animals given lindane at the rate of 100 p.p.m. in their food for 84 days allowed screw-worms to develop *in vitro*, although the animals remained toxic to horn-flies for a further 21 days after feeding ceased. It is possible that in some way the toxicants become involved

in the sebaceous secretory mechanism. Against this, lindane at least has not been found consistently in the hair of injected cattle (method sensitive to 10 μg lindane in the hair from 12 sq. in. cattle hide) and it could only be detected for portion of the period over which toxic effects were exerted on ticks. It is also possible that a repellent effect operates, since Garnham (1947) reported that mosquitoes were reluctant to feed on animals treated with BHC.

After injection it is possible to rub the solutions for considerable distances around the body of the animal in the subcutis, and it is likely that skin and muscle movements assist the distribution of the newly injected material over a considerable area of the body.

The experiments throw some light on the mode of action of toxicants already in widespread use for cattle tick control. Spence (1952*b*), discussing the action of BHC in killing scab mites present in untreated areas inside the ears of dipped sheep, suggested that absorption and excretion of BHC had occurred. Comparable action by lindane and dieldrin against cattle ticks was demonstrated in the inunction experiments, and it seems possible that these substances exert their effects partly by this mechanism following dipping. Amounts absorbed during normal acaricidal treatment are doubtless small, but may be sufficient to affect larvae attaching to untreated regions. By contrast, the experiments showed that DDT, toxaphene, malathion, diazinon, isodrin, and endrin, at the dosages employed, exerted no systemic effect at all on the ticks, in spite of their known high contact toxicity to larvae. A similar observation was made by Knipling *et al.* (1948).

Table 1 shows that there was a wide range of variation in systemic effects between animals within groups, and there were certain differences between the performance of the same toxicant in different experiments. Lindquist *et al.* (1944) reported that successive experiments with the oral administration of DDT and pyrethrum actually gave conflicting results. Knipling *et al.* (1948) found the systemic effects of inunction to be erratic, and this is confirmed by experiments by the present author subsequent to that reported herein. Further work is required on the factors responsible for the variability of results.

The demonstration that local application of small quantities of concentrated toxicant solutions affects ticks over the entire body surface may ultimately have practical applications in cattle ectoparasite control by self-treatment devices such as those described by Rogoff and Moxon (1952) and others.

Subcutaneous injections, however, are not considered to afford an immediate practical method of tick control, as studies are required on the hazards to human health resulting from toxic substances remaining in the tissues of animals after slaughter, the safety of the technique for the animals, and other matters.

Gladenko (1954) reported a musty smell in the fat of cattle 60 days after oral administration of BHC, but this was probably not due to the gamma isomer. No symptoms of acute toxicosis developed in animals which ate the flesh of beasts killed in an advanced stage of BHC poisoning. McGregor *et al.* (1955) showed by biopsies that considerable quantities of lindane, dieldrin, and aldrin remained in the tissues of cattle 1 month after subcutaneous injections ceased, and that dieldrin and aldrin were still present even after 4 months. Claborn *et al.* (1953) found that the meat contamination of animals eating pesticides was lowest with toxaphene, followed in order by DDT, chlordane, dieldrin, aldrin, and BHC. In experiments conducted by Ely *et al.* (1954), 11-14 per cent. of aldrin fed to dairy cows was excreted in the milk.

The safety of the technique to the animals is also important. Lindquist *et al.* (1953) reported the deaths of three calves injected respectively with 10, 25, and 50 mg lindane/kg. McGregor *et al.* (1955) injected groups of four bovines subcutaneously with lindane, dieldrin, and aldrin at 28-day intervals. All animals receiving 25 mg dieldrin/kg and 50 mg lindane/kg survived six treatments, but aldrin injections were discontinued when one animal died after the third injection at the rate of 50 mg/kg. By contrast, cattle given 25 mg/kg of lindane, dieldrin, and aldrin orally in a 5 per cent. emulsion died within 5 hours. During the experiments described in the present paper, mortality of experimental animals occurred only after the injection of endrin, but no others showed signs of toxicosis. Calves produced by a number of cows either during or soon after the experiments were normal.

Peanut oil, the vehicle used in the present series of experiments, is also relatively expensive, and the quantities of toxicants used per beast are in excess of the amounts normally carried out by animals after immersion in a dip. Once suitable toxicants and vehicles have been discovered, however, this may be offset by the avoidance of major wastages involved in periodically recharging vats after cleaning, or changing the toxicant to cope with resistant ticks.

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GENETIC PARAMETERS ASSOCIATED WITH CHARACTERS AFFECTING EGG PRODUCTION IN THE DOMESTIC FOWL

I. HERITABILITY OF TOTAL EGG PRODUCTION DURING THE PULLET YEAR

By B. L. SHELDON*

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Summary

Egg production records of White Leghorn pullets hatched at the Poultry Research Centre, Werribee, Vic., during 1947 to 1950 inclusive have been analysed both on a hen-housed (Production Index) and a survivor basis. Estimates of heritability were obtained by two methods: (1) by the analysis of components of variance between families of full sibs and half-sibs; (2) by estimating the intra-sire regression of the mean performance of offspring on dam's performance. Heritability, in the narrow sense, of Production Index during the pullet year is shown to be 25-30 per cent. with a reasonably low standard error, while heritability of survivors' production is slightly lower but not significantly so. Possible reasons are advanced for the low estimates of heritability of survivors' production.

I. INTRODUCTION

The earlier literature on the genetic parameters of production characters in the fowl was fully reviewed by Lerner (1950). The character with which this study is concerned is that of total egg production during the first laying year. While evidence from several sources suggested that a heritability of 30 per cent. for the production of birds surviving the first laying year was quite general, only one estimate of the heritability of hen-housed production (Production Index) was available. It placed the heritability of this latter character in the University of California flock as low as 5 per cent. (Lerner 1950). A later analysis of the same flock based on the period 1933-1948 led to an estimate of about 15 per cent. for the Production Index (Dempster, Lerner, and Lowry 1952).

More recently King and Henderson (1954) have estimated that the heritability of the Production Index in the first laying year in a commercial flock was about 20 per cent., while that of survivors' production was about 30 per cent.

This paper presents estimates of the heritability of these characters in the flock of the Poultry Research Centre at Werribee, Vic. It deals with total egg production during the pullet year and is a preliminary analysis of the situation in this flock.

II. MATERIALS AND METHODS

The records of production of all non-inbred White Leghorns hatched from 1947 (foundation flock) to 1950 inclusive have been used in this

* Animal Genetics Section, C.S.I.R.O., University of Sydney.

study. Pullet-year production has been measured over a period from first egg till March 31 following their first 12 months of life. This period has been described by Skaller (1954) as the Standard Pullet Year. Details of the management of this flock have also been presented by Skaller (1955). The main features of importance for the present paper are that hatching took place each year over a period of 9-11 weeks in the spring (August-September) and that birds were housed at random before laying began. All pullets hatched in 1947 were housed in trap-nest sheds on deep litter, while those hatched in 1950 were all housed in individual battery cages. Pullets hatched in 1948 and 1949 were distributed over both types of housing system.

Skaller (1954) has shown that the Standard Pullet Year production is independent of hatching date, when hatching is confined to the short period mentioned above, so differences in hatching date have not been considered in the following analyses.

Heritability has been estimated by two different methods: (1) by the analysis of the components of variance in the production of full and half-sisters; (2) by estimating the intra-sire regression of mean performance of offspring on dam's performance. The samples differed somewhat between the two methods. For example, in the data collected for use with the regression method, some groups in which proven sires or dams had been selected were included. These were omitted from the variance analysis. This will explain the apparent discrepancies in the number of degrees of freedom given for the two methods in the following section. Standard errors of the heritability estimates made from analysis of components of variance were computed by the approximate method for intra-class correlations (Fisher 1946).

III. Results

Estimates of the heritability of the Production Index during the Standard Pullet Year are given in Table 1. Because of the small numbers involved the individual estimates for each generation have a high standard error. Three methods have therefore been used to obtain pooled estimates with a relatively low standard error: (*a*) the analyses (variance components or regression) for each year were pooled; (*b*) individual heritability estimates were weighted by the number of degrees of freedom; (*c*) estimates were weighted by the inverse of their variances. Results obtained by these three pooling methods are in very close agreement within each type of heritability determination (h_S^2 from sires' component, h_D^2 from dams' component, or $h_{P.O.}^2$ from parent-offspring regression), and there is a fairly close agreement between h_S^2, h_D^2, and $h_{P.O.}^2$. The lowest pooled estimates given in Table 1, those for h_S^2, indicate that the heritability of Production Index is approximately 25 per cent.

Since the Production Index is so closely connected with flock mortality after the housing of mature pullets, it is necessary to consider

TABLE 1

ESTIMATES OF HERITABILITY OF PRODUCTION INDEX

Year of Hatch	Sires' Component		Dams' Component		Joint Term	Parent-offspring Regression	
	h^2_S	D.F.*	h^2_D	D.F.	$h^2_{(S+D)/2}$	$h^2_{P.O.}$	D.F.
1947	24 ± 11.2	12	16 ± 11.8	289	20	22 ± 11.9	288
1948	18 ± 9.4	25	71 ± 17.0	136	44	36 ± 27.8	82
1949	36 ± 17.4	15	32 ± 20.9	89	34	46 ± 16.2	138
1950	26 ± 14.0	12	28 ± 16.3	68	27	28 ± 17.9	107
(*a*) Pooled	24 ± 5.8	64	36 ± 7.9	582		30 ± 8.2	
(*b*) Weighted (by D.F.)	25	64	33			30	
(*c*) Weighted (by inverse of variance)	23		32			30	

* Degrees of freedom.

TABLE 2

ESTIMATES OF HERITABILITY OF SURVIVORS' PRODUCTION

Year of Hatch	Sires' Component		Dams' Component		Joint Term	Parent-offspring Regression	
	h^2_S	D.F.	h^2_D	D.F.	$h^2_{(S+D)/2}$	$h^2_{P.O.}$	D.F.
1947	3 ± 4.3	12	136 ± 17.4	251	70	28 ± 12.4	233
1948	44 ± 16.2	25	18 ± 17.4	122	31	29 ± 20.0	58
1949	24 ± 16	15	26 ± 26.2	83	25	8 ± 18.0	109
1950	34 ± 18.1	12	45 ± 19.3	64	40	18 ± 17.0	80
(*a*) Pooled	18 ± 5.2	64	74 ± 10.6	520	46	22 ± 8.2	
(*b*) Weighted (by D.F.)	30	64	80	520		22	
(*c*) Weighted (by inverse of variance)	8		62			22	

the mortality figures in conjunction with the above heritability estimates. The percentage mortalities in the White Leghorn pullet flock for the years of hatch 1947 to 1950 inclusive were respectively 33, 11, 13, and 11 per cent. Only in the 1947 flock was mortality above normal. This was due to a high incidence of cannibalism among the pullets not housed in battery cages, which accounted for about 75 per cent. of all deaths during that year.

Estimates of the heritability of production by pullets surviving the Standard Pullet Year are given in Table 2. The pooled estimates of h_S^2 and $h_{P.O.}^2$ show the heritability of survivors' production to be about 20 per cent., apparently lower than that of the Production Index but not significantly so. The low figure obtained for h_S^2 after pooling the individual estimates, weighted according to the inverse of their variances, is due to the large weighting given to the estimate for 1947.

IV. Discussion

On the basis of previous experience, the heritability of survivors' production was expected to be considerably higher than that of the Production Index, but it has not been found to be so here. A possible reason for this is that in the years of hatch 1948 and 1949 about 10 per cent. of birds were culled up to 3 or 4 months before the end of the Standard Pullet Year, when they had stopped laying, because it was assumed they would not resume laying during the remainder of the Standard Pullet Year. This was done to make room for incoming birds. It can be argued that a closer approximation to heritability of survivors' production would have been obtained by including the culled pullets in the survivors' analysis, but either method of handling these data could lead to an appreciable error. For this paper the culled birds were omitted from the analysis of survivors' production. A further reason for the lack of difference between the heritabilities of hen-housed and survivors' production is undoubtedly the relatively low mortality rate during the years considered here. The fact that h_D^2 is rather higher than h_S^2 in most instances has been observed in previous studies, and the possible reasons have been discussed by King and Henderson (1954). A conclusion as to the precise reason will not be possible until adequate tests have been carried out.

Heritability estimates from the intra-sire regression of offspring on dam were obtained without any weighting for the number of offspring in each dam's family. The unweighted mean of the offspring was used, which leads to an estimate slightly on the low side (Kempthorne and Tandon 1953). Taking h_S^2 and $h_{P.O.}^2$ as the estimates most closely representing heritability in the narrow sense, that is, the fraction of the variance which is additively genetic, the conclusion can be drawn that heritability of Production Index during the pullet year for this flock is of the order of 25-30 per cent. The applicability of this estimate to other

flocks is to a certain extent doubtful, especially when one considers the low mortality figures and the fact that management procedures have probably reduced the environmental portion of the variance. As already explained, there is some doubt about the estimate of the heritability of survivors' production. Its heritability here is not significantly different from that of Production Index, but to judge from estimates made in other flocks, it is probable that h^2 for survivors would be greater than for Production Index, if a suitable and large sample were available for analysis.

The heritability of Production Index during the period stated is as high as 30 per cent., and h^2 for shorter periods of the laying year should be even higher (Lerner 1950; King and Henderson 1954). The periods of interest to this Centre are the 500 days or 72 weeks test, and the part-winter period to May 31 in the first year of life, on which latter most of the selection work is based.

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GENETIC PARAMETERS ASSOCIATED WITH CHARACTERS AFFECTING EGG PRODUCTION IN THE DOMESTIC FOWL

II. HERITABILITY OF EGG PRODUCTION FOR TWO PART-ANNUAL PERIODS OF MEASUREMENT AND THE GENETIC CORRELATION BETWEEN THEM

By J. A. Morris*

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Summary

Estimates of important genetic parameters have been obtained from an experimental flock using data collected over a period of 3 years. A total of 1784 progeny, obtained from 42 sires and 383 dams, supplied records for analysis. The information was mainly extracted using the analysis of variance and covariance from which estimates of genetic variance and covariance were obtained.

Pooling the results within years and, where applicable, within breeding groups, estimates of heritability were obtained as follows: Production Index: part period, 32 per cent.; 72 weeks, 31 per cent. Survivor Index: part period, 33 per cent.; 72 weeks, 33 per cent. The low mortality rate should be borne in mind when comparing the estimates for the Production Index with those obtained by other workers. The estimated genetic correlation between the Production Index for these two periods was 0.72.

The magnitude of the heritability estimates indicates the presence of a considerable amount of additive genetic variation. The proportion of this type of genetic variation has probably been increased by the minimizing of environmental variation effected by the randomization of birds over the housing space and, in the case of the part period of recording, by the corrections applied for hatching date influence.

Early selection, based on partial records, has been shown to be approximately 1½ times as effective in producing genetic improvement as selection based on the full production.

I. Introduction

Numerous reports on the heritability of productive characters of the domestic fowl are available in the literature and King (1953) has reviewed them in some detail. Part I of this series (Sheldon 1956) contains more recent estimates of heritability of total egg production during the pullet year with a value of 25-30 per cent. for the Production Index and a slightly lower value for survivors.

King (loc. cit.) reported higher estimates for heritability of both the Production Index and the survivors for the short period of measurement terminating on January 1 than for total production. Lerner and Cruden (1948) had previously made similar observations on their

* Division of Animal Health and Production, C.S.I.R.O., Poultry Research Centre, Werribee, Vic.

production-bred flock; they suggested that selection might take place on a part record basis, thus considerably reducing the interval between generations. In this connexion, they estimated the genetic correlation between part-period (i.e. first egg up to January 1) and total production (all eggs recorded to September 30 of the year following hatch) on a survivor basis as being 0.74. Dempster and Lerner (1947) estimated the analogous correlation for the Production Index to be 0.75.

The present investigation provides further information on these genetic parameters using data from a large experimental population, details on which are supplied in part I.

II. Materials and Methods

The data used for this study were obtained from the same flock as those used in part I. Information was extracted from three consecutive years of records commencing with the pullets hatched in 1947, which represented the parental generation for the subsequent breeding programme. All available records were used for this particular year, whereas in the following two years some groups were not considered because of the lack of randomness associated with the matings. The effect of culling towards the end of the pullet laying year mentioned in part I was unimportant as far as this study was concerned, for the 72-week period of production had invariably been completed before such culling took place.

In connexion with the part period of production, which is defined to extend from the first egg until May 31, corrections were applied in an endeavour to compensate for hatching date influence as well as for the reduced period of testing for successive hatches. All records were adjusted to a common hatching date, usually the median hatch but always to a similar date period of the year, the corrections being based on linear trends. Such corrections require the assumption of absence of interaction of genic effects with hatching date. Corrections were not applied to the 72-week records as, after an examination for possible hatching date influences, it was concluded that such effects were negligible over the range of hatching period commonly used at this Centre.

Considerable trouble was experienced in the first year with feather and vent picking. Approximately 20 per cent. of all pullets housed died during their first year as a result of such cannibalism. In many cases records would be complete for the part period of measurement and incomplete for 72 weeks. All such cannibalized birds were eliminated from the analysis for those periods for which their records were incomplete.

Finally, only offspring resulting from cockerel by pullet matings were considered. This restriction was made in order to maintain a state of randomness in the matings. Hence, cases of genetic assortative matings as well as matings involving proven sires and dams were eliminated from the analyses.

III. Experimental Results

Since the numbers of observations available for analysis differed for the two characters, an indication of the size of the sample is presented in Table 1. It will be noticed that the principal discrepancy occurs in 1948, when many more records were available for the part period than for 72 weeks. As pointed out previously, cannibalism was responsible for many premature deaths in this year and although these birds had reliable records for the part period, it was not considered wise to include them in the 72 weeks' analysis. Such an omission could lead to small inaccuracies in the estimation of the parameters concerned, since it is not unreasonable to assume that some of the birds lost in this way were suffering from

Table 1

size of sample

Figures in brackets indicate numbers available for part-period analysis. Unbracketed figures refer to 72 weeks' analysis

Index	Year of Hatch			Total
	1947	1948	1949	
Production Index:				
Sires	(12) 12	(20) 20	(10) 10	(42) 42
Dams	(219) 198	(103) 103	(61) 61	(383) 362
Progeny	(930) 763	(529) 518	(325) 319	(1784) 1600
Survivor Index:				
Sires	(12) 12	(20) 20	(10) 10	(42) 42
Dams	(218) 189	(103) 100	(61) 58	(382) 347
Progeny	(913) 694	(524) 488	(317) 287	(1754) 1469

prolapse or were otherwise unhealthy, and hence very susceptible to the malicious intents of their neighbours. Against this, it might be argued that the larger percentage of these birds died before the termination of the 72-week period not because of a genetic susceptibility to early death, but more because of mischance. The small number of discrepancies in the later years can be attributed to incomplete records due, for instance, to a bird being missing from its cage or shed for a period of time and in a few cases to accidental death such as strangulation.

The mean production and phenotypic variances of the flock under study are given in Table 2. Because of the rather low mortality prevailing in the flock at the time concerned, the mean Production Index for the three years does not differ much from the mean Survivor Index. The mortality percentages for the part period for successive years were 1.8, 1.0, and 2.5 respectively whilst corresponding figures for the 72 weeks were 9.0, 5.8, and 10.0. Total mortality over all years was 1.7 per cent. for the part period and 8.2 per cent. for 72 weeks.

Heritability was estimated for both the Production Index and survivors by variance component analysis. The method of intra-sire regression of daughters' mean production on dam's production was used for estimating the heritability of the Production Index only. A minimum

TABLE 2
MEAN PRODUCTION AND PHENOTYPIC VARIANCES OVER YEARS

Index	Year of Hatch		
	1947	1948	1949
Production Index			
Part period:			
Mean	43.3	35.3	35.6
Variance	242.9	301.1	358.4
72 Weeks:			
Mean	150.8	158.3	151.0
Variance	2814	2275	3122
Survivor Index			
Part period:			
Mean	43.8	35.6	36.4
Variance	222.7	291.7	340.9
72 Weeks:			
Mean	159.0	163.6	159.9
Variance	1958	1702	2189

of two daughters was required for a dam's progeny to be included in the analysis of variance, whereas in the intra-sire regression of daughter on dam a single daughter sufficed for inclusion. Heritability estimates have been obtained for the part-period and for the 72 weeks' production. The

TABLE 3
ESTIMATES OF HERITABILITY: PART PERIOD

Source of Estimate	Production Index			Survivor Index		
	1947	1948	1949	1947	1948	1949
(i) Sire component	22.0	41.1	17.6	26.8	41.7	13.3
(ii) Dam component	44.4	38.1	32.4	46.8	34.8	29.3
(iii) Joint term	33.2	39.5	25.0	36.8	38.2	21.3
(iv) Daughter-dam regression	—	2.4	12.8	—	—	—

genetic correlation between these two characters has been estimated only for the Production Index, using the method of cross correlation (Hazel 1943) and variance-covariance analysis (Hazel, Baker, and Reinmiller 1943).

Estimates of heritability are set out in Tables 3 and 4 for the two periods under study, detailed for the three years. It is interesting to note

the very low values obtained for the part period by the method of regression. King (1953) obtained similar results. The discrepancy seems to arise from the fact that the parents are all survivors, since to be parents they must have survived well beyond the period ending May 31, which date actually terminates the part period. The position is not so marked in the case of 72 weeks, where the parents are only required to be

TABLE 4

ESTIMATES OF HERITABILITY: 72 WEEKS

Source of Estimate	Production Index			Survivor Index		
	1947	1948	1949	1947	1948	1949
(i) Sire component	11.2	16.4	41.0	16.3	25.8	28.8
(ii) Dam component	32.8	58.1	40.0	54.5	42.2	22.6
(iii) Joint term	22.0	37.2	40.5	35.5	34.0	25.7
(iv) Daughter-dam regression	—	22.0	29.0	—	—	—

survivors up to breeding time. The values obtained can scarcely be regarded as valid estimates of heritability, particularly in the case of the part period, when daughters' mean Production Index is regressed on dam's survivor value. However, because of the low mortality associated with the part period, the estimates obtained may not be seriously biased.

TABLE 5

ESTIMATES OF HERITABILITY: ANALYSIS POOLED WITHIN YEARS

Source of Estimate	Part Period		72 Weeks	
	Production Index	Survivor Index	Production Index	Survivor Index
(i) Sire component	27.3	29.0	18.9	20.7
(ii) Dam component	37.4	36.2	42.6	44.8
(iii) Joint term	32.4 ± 5.04	32.6 ± 5.04	30.8 ± 5.36	32.8 ± 5.74
(iv) Daughter-dam regression	10.0	—	26.0	—

The yearly analyses have been pooled to give an overall within year estimate of heritability and these figures are presented in Table 5. Considering the values obtained from the full-sister correlation, that is, the estimate obtained from both the sire and dam component of variance, there seems to be little difference between the heritabilities for the two periods on either index. A figure somewhere in the vicinity of 30 per cent. would be equally applicable to each case. In the case of 72 weeks' Production Index, the value obtained from the regression, 26 per cent., might strengthen this belief.

The selection of breeders on the basis of part-period performance is justified by the assumption that its genetic correlation with the total period of pullet production is high. The amount of progress per year in the 72-week period to be expected from family selection based on 72 weeks' records is

$$\Delta G_{F\ (\text{direct})} = \frac{i\sigma_{fa_F} h^2_{fa_F}}{2}. \qquad \text{(See Lerner 1950.)}$$

Analogously, the amount of yearly progress in 72 weeks' production to be expected by selection on the part period would be

$$\Delta G_{F\ (\text{indirect})} = \frac{ir_{G_P G_F}\sigma_{G_F} h^2_{fa_P}\sigma_{fa_P}}{\sigma_{G_P}},$$

the subscripts F and P referring to 72 weeks and part periods respectively. The amount of progress in 72 weeks' production, per year, resulting from selection on part records, as compared to that expected if the selection were based on the 72 weeks' production, would be given by

$$\frac{\Delta G_{F\ (\text{indirect})}}{\Delta G_{F\ (\text{direct})}} = \frac{2r_{G_P G_F} h_{fa_P}}{h_{fa_F}}.$$

We have seen from Table 5 that the heritability of the part period Production Index is very similar to that of 72 weeks, both being approximately 30 per cent. The ratio h_{fa_P}/h_{fa_F} can then be written as unity, and the ratio of indirect to direct selection, in so far as genetic gain per year is concerned, can be written as $2r_{G_P G_F}$; this applies equally in the case of selection on the performance of individuals, or on that of families on the appropriate combination of individual and family performance.*

We should now examine the results obtained from the data for the genetic correlation. These are set out in Table 6. It will be seen that the estimates are quite high, the value of 0.72 obtained from a combination of the sire and dam components of variance and covariance from the pooled analysis within years agreeing very favourably with the only previous estimate of 0.75 as reported by Dempster and Lerner (1947). The pooled value resulting from the method of cross correlation is somewhat lower at 0.56, but this may not be as reliable as the other estimate, being based on two years' data as against three years for the analyses of variance and covariance. In addition, since the expression yielding the genetic correlation contains in the numerator the correlation between offspring's 72 weeks' production and dam's part-period production, and

* Sequential selection, where previously selected birds may be subsequently selected again when more information becomes available concerning their breeding value, is outside the scope of this paper.

in the denominator the correlation between offspring's part-period and dam's part-period production, it is difficult to see how the previously mentioned fact that all dams are survivors for the part period would affect the ultimate estimate. Taking an average, weighted according to the degree of freedom, of all the estimates resulting from the analysis pooled over years, the genetic correlation turns out to be 0.61. Because of the generally higher values obtained for each of the years, as evidenced in Table 6, and the unreliability of the cross correlation method for the Production Index, this value of 0.61 should probably be regarded as more of a lower limit than an average value.

TABLE 6

GENETIC CORRELATION BETWEEN PRODUCTION INDEX OF PART PERIOD AND PRODUCTION INDEX OF 72 WEEKS

Year	Method of Estimation			
	Sire Term	Dam Term	Joint Term	Cross Correlation
1947	0.92	0.39	0.54	—
1948	0.85	0.88	0.82	0.40
1949	1.02	0.75	0.86	0.80
Pooled	0.90	0.62	0.72	0.56

Estimate obtained by weighting individual estimates from pooled analysis according to degrees of freedom: 0.61

IV. DISCUSSION

King (1953) appears to be the first worker who made allowance for the effect of hatching date in estimating the heritability of productive characters in the domestic fowl. He regarded this as being the principal explanation for the higher heritability values obtained for the Production Index than observed by Lerner and his co-workers previously. Part-period production records have always been corrected for hatching date at this Centre and the values submitted for heritability in this paper are based on analyses of the corrected records. Thus it is not unreasonable to assume that the hatch effect has been to a large extent eliminated. Our results agree very well with those of King in that heritability of the Production Index seems to be much higher than at first thought. This, of course, could be a peculiarity of the flock under study. Since the mortality of our flock was very low for the years concerned, one would not expect the heritability of the Production Index to differ a great deal from that of the survivors and it does not. This would particularly apply to the part period when the mortality was around the 2 per cent. mark. Lerner and Hazel (1947) and Dempster, Lerner, and Lowry (1952) reported much lower values for the heritability of the Production Index. However, mortality in their flock was considerably higher than in ours. The latter authors reported an average mortality for the period 1933-1940 of 35

per cent. and for the period 1941-1948 of 22 per cent. Apparently estimates of the heritability of the Production Index are not particularly enlightening unless accompanied by flock mortality figures. The rather large values obtained for the Production Index in the present study may not be representative of what might be found on the average commercial farm, where mortality would usually be considerably in excess of our figure of 8.2 per cent. In addition, since all birds are randomized throughout available housing space, it might be expected that environmental variance would be somewhat reduced, with a resultant increase in heritability.

In most cases the estimate of heritability obtained from the dam component of variance was in excess of that obtained from the sire component. King observed this in his data and provided three possible explanations as to its occurrence. His assertion that the probably greater selection intensity practised on the sires could account for the discrepancy would not apply to our data, for in the developing stages of our breeding programme the sires would not have been subjected to any higher selection pressure than the dams. The possibility of a maternal effect or sire-dam interaction cannot be discounted but the present data are inadequate to establish such effects. Since the dam component of variance is known to contain one-quarter of the dominance deviations and since the component is multiplied by the factor four in obtaining heritability, it is most likely that heritability is over-estimated because of this.

Lerner and Cruden (1948) and King (1953) reported that heritability declined as the laying season progressed. The decline in King's data was consistent though it only applied to Lerner and Cruden's Production Line, the other lines examined as well as the pooled analysis not giving a similar trend. There does not appear to be any conclusive evidence from our results that heritability for the part period is in excess of that for 72 weeks. The joint estimate from the pooled analysis indicated a heritability of around 30 per cent. for both periods, the actual values being 32.4 per cent. for the part period and 30.8 per cent. for 72 weeks. Very similar results were obtained for survivors, heritability for the part period being 32.6 per cent. and for 72 weeks 32.8 per cent. Even if a fairly large difference between the respective heritabilities did exist in favour of the part period, it would play a minor role in determining the efficacy of family selection on the part period (as against selection on the 72 weeks basis) compared to the effect of halving the generation interval. If individual selection were practised the effect of a higher heritability for the part period would be much more important. Lerner and Cruden (1948) suggest as an explanation for the higher heritability for the part period found in their Production Line the fact that selection was practised mostly on complete pullet records, with a consequent greater reduction in genetic variability associated with that period as compared with the part period. A large proportion of our data refer to an unselected

flock, and for the remainder selection was rather weak and always based on the part period. One would not have expected any difference, at the present stage, because of selection. A lower heritability for the 72 weeks could have logically been expected on the grounds of an increased exposure to environmental vicissitudes. However, such does not appear to have been the case.

As was seen previously, the genetic correlation between the two periods plays a very significant role in determining how effective early selection can be. In the case where the two heritabilities seem to be of approximately the same magnitude, early selection provides $2r_{G_P G_F}$ times as much genetic gain as does selection on the full record. From our data, 0.72 would be the best estimate of the genetic correlation and hence early selection should provide approximately 1.44 times as much genetic gain as would selection on the full record. Considering purely family selection and using the heritability values of King (1953), 0.34 for the part period and 0.20 for 365 days' production, for families of 40 half-sisters the ratio h_{fa_P}/h_{fa_F} would be 1.079, and for families of 10 full sisters the ratio would be 1.13. The effect of this increased heritability for the part period would be to increase the genetic gain for half-sister selection from 1.44 to (1.44) (1.079) = 1.55, and for full-sister selection from 1.44 to (1.44) (1.130) = 1.63, times that which would be obtained using similar systems of selection but based on the full period of measurement. Therefore, early selection based on part records should provide advancement at a rate of approximately $1\frac{1}{2}$ times that to be expected if selection were confined to the full period.

The estimates of the genetic correlation obtained by the method of cross correlation (and this also applies to the estimates of heritability of the Production Index obtained by the intra-sire regression of daughter on dam) are not strictly valid, since all dams must be survivors for a period well beyond May 31; they are included because owing to the low mortality in the part period it is felt that they may not be seriously biased.

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INDEX

PAGE

Andrew, W. D., and Neal-Smith, C. A.—
The Effect of Associate Perennial Grasses on a Simple Pasture Mixture of Phalaris and Subterranean Clover 367

Angus, D. E.—
Spring Frost Damage to Vines 163

Antcliff, A. J., Webster, W. J., and May, P.—
Studies on the Sultana Vine. IV. A Pruning Experiment with Number of Buds per Vine Varied, Number of Buds per Cane Constant .. 401

Apricots, Brown Rot of. I, II 504, 516

Bailey, S. W.—
Air-tight Storage of Grain; its Effects on Insect Pests. II. *Calandra oryzae* (Small Strain) 7

Barley, K. P., and Hutton, J. T.—
Effects of a Lowland Rice Crop and of Gypsum on a Saline Alkali Clay 110

Biddiscombe, E. F., Hutchings, R. J., Edgar, G., and Cuthbertson, E. G.—
Grazing Management of Natural Pasture at Trangie, New South Wales 233

Binet, F. E.—
See Robinson, T. J. 345

PAGE

Black, J. N.—
The Influence of Seed Size and Depth of Sowing on Pre-emergence and Early Vegetative Growth of Subterranean Clover (*Trifolium subterraneum* L.) .. 98

Blackburn, G.—
See Jackson, E. A. 20

Blackmore, A. V.—
Time and Temperature as Factors in the Dispersion of Soil Crumbs in Water 554

Boophilus microplus (Canestrini) (cattle tick), Infestations, Effect of Self-licking by Cattle on 227

Boophilus microplus (Canestrini), Systemic Effects of Chlorinated Hydrocarbons against 608

Boophilus microplus (Canestrini), Toxaphene-resistant, in Queensland 211

Brown Rot of Apricots caused by *Sclerotinia fructicola* (Wint.) Rehm. I, II .. 504, 516

Bouma, D.—
Studies in Citrus Nutrition. II. Phosphorus Deficiency and Fruit Quality 261

Brougham, R. W.—
Effect of Intensity of Defoliation on Regrowth of Pasture 377

PAGE

Bull, L. B., Dick, A. T., Keast, J. C., and Edgar, G.—
An Experimental Investigation of the Hepatotoxic and other Effects on Sheep of the Consumption of *Heliotropium europaeum* L.: Heliotrope Poisoning of Sheep 281

Butler, B. E., and Hutton, J. T.—
Parna in the Riverine Plain of South-eastern Australia and the Soils thereon . . 536

Calandra oryzae L. (rice weevil), Effects of Air-tight Storage of Grain on 7

Carr, D. J., and Eng, Kok Ng—
Experimental Induction of Flower Formation in Kikuyu Grass (*Pennisetum clandestinum* Hochst. ex Chiov.) 1

Carter, H. B.—
See Daly, R. A. 76

Cattle, Heat Tolerance, Experimental Study of 469

Cattle, Sweat Glands in. I . . 482

Cattle Tick, Infestations, Effect of Self-licking by Cattle on 227

Cattle Tick, Systemic Effects of Chlorinated Hydrocarbons against 608

Cattle Tick, Toxaphene-resistant, occurring in Queensland 211

Chlorinated Hydrocarbons, as Systemic Acaricides against the Cattle Tick 608

PAGE

Citrus Nutrition, Studies in. I, II 248, 261

Clarke, A. R. P.—
See Jackson, E. A. 20

Clay, Saline Alkali, Effects of a Lowland Rice Crop and of Gypsum on 110

Copper, Distribution in Subterranean Clover Seedlings in Solution Culture 495

Copper-molybdenum-sulphate Interaction, in Induction of Ovine Hypocuprosis 45

Cox, J. E.—
See Martin, A. E. . . 169, 184

Cuthbertson, E. G.—
See Biddiscombe, E. F. . . 233

Daly, R. A., and Carter, H. B.—
Fleece Growth of Young Lincoln, Corriedale, Polwarth, and Fine Merino Maiden Ewes Grazed on an Unimproved Paspalum Pasture 76

Darling Downs (Qld.), Nitrogen Studies on Black Soils from. I, II 169, 184

Davern, C. I.—
See Morley, F. H. W. . . 388

Dowling, D. F.—
An Experimental Study of Heat Tolerance of Cattle . . 467

Dick, A. T.—
See Bull, L. B. 281

Doig, Alison G.—
See Robinson, T. J. 345

Drosophila melanogaster, Effect of Cold Treatment on Mutation in 566

PAGE

Edgar, G.—
See Biddiscombe, E. F. .. 233
See Bull, L. B. 281
Egg Production, in Domestic Fowl, Genetic Parameters Associated with Characters Affecting. I, II 625, 630
Eng, Kok Ng—
See Carr, D. J. 1
Ewes, Anoestrous, Effects of Progesterone and Pregnant Mare Serum Administration on. I, II 591, 601
Ewes, Fleece Growth of Young Lincoln, Corriedale, Polwarth, and Fine Merino, Grazed on Unimproved Paspalum Pasture 76

Fat Lamb Studies in Victoria. I 345
Fleece Growth, of Young Maiden Ewes Grazed on Unimproved Paspalum Pasture 76
Flower Formation in Kikuyu Grass, Experimental Induction of 1
Fowls, Domestic, Genetic Parameters Associated with Characters Affecting Egg Production in. I, II .. 625, 630
Frost Damage, to Vines, in Spring 163
Fry, P. R.—
See Yen, D. E. 272

Gates, C. T.—
See Williams, R. F. 248
Gene Dosage, in *N*-type Sheep 447
Grain, Air-tight Storage, Effects on Insect Pests. II 7

PAGE

Grasses, Perennial, Effect on Phalaris–Subterranean Clover Pasture Mixtures .. 367
Grasslands, Semi-arid, Plant-soil Relationships in 127
Grylls, N. E.—
See Hutton, E. M. 85
Gypsum, and Lowland Rice Crop, Effects on a Saline Alkali Clay 110

Hayman, R. H.—
See Nay, T. 482
Heliotropium europaeum L. (heliotrope), Hepatotoxic and other Effects on Sheep Consuming 281
Hutchings, R. J.—
See Biddiscombe, E. F. .. 233
Hutton, E. M., and Grylls, N. E.—
Legume "Little Leaf", a Virus Disease of Subtropical Pasture Species .. 85
Hutton, J. T.—
See Barley, K. P. 110
See Butler, B. E. 536
Hypocupraemia and Hypocuprosis, Ovine, Copper-molybdenum-sulphate Interaction in Induction of .. 45

Insemination, Artificial, of Merino Sheep Following Synchronization of Oestrus and Ovulation by Progesterone Injected alone and with Pregnant Mare Serum Gonadotrophin 194
Jackson, E. A., Blackburn, G., and Clarke, A. R. P.—
Seasonal Changes in Soil

PAGE

Salinity at Tintinara, South Australia 20

Jones, G. B.—
See Riceman, D. S. 495

Keast, J. C.—
See Bull, L. B. 281
Kikuyu Grass, Induction of Flower Formation in 1
Kuchel, R. E.—
See Lee, H. J. 333

Lambs, Birthcoat of, and Adult Fibre Diameter 152
Lamond, D. R.—
See Raeside, J. I. 591, 601
Leaf Growth and Composition, in Citrus 248
Legume "Little Leaf", a Virus Disease of Subtropical Pasture Species 85
Lee, H. J., Kuchel, R. E., and Trowbridge, R. F.—
The Aetiology of Phalaris Staggers in Sheep. II. The Toxicity to Sheep of Three Types of Pasture Containing *Phalaris tuberosa* L. . . 333
Lockart, L. W.—
Birthcoat of Lambs and Adult Fibre Diameter . . 152
The Reaction of Primary and Secondary Wool Fibres to Changes in Nutrition . . 147
Variation in Wool Production per Unit Area of Skin 158

McCance, I.—
See Watson, R. H. 574
Malachite Green, Effect on Potato Viruses X and Y . . 428

PAGE

Martin, A. E., and Cox, J. E.—
Nitrogen Studies on Black Soils from the Darling Downs, Queensland. I. Seasonal Variations in Moisture and Mineral Nitrogen Fractions 169
II. Nitrifying Activity of Subsurface Horizons 184
May, P.—
See Antcliff, A. J. 401
Molybdenum, Interaction with Copper and Sulphate in Induction of Ovine Hypocuprosis 45
Morley, F. H. W.—
Selection for Economic Characters in Australian Merino Sheep. VII. Interactions between Genotype and Plane of Nutrition . . 140
Morley, F. H. W., and Davern, C. I.—
Flowering Time in Subterranean Clover 388
Morris, J. A.—
Genetic Parameters Associated with Characters Affecting Egg Production in the Domestic Fowl. II. Heritability of Egg Production for Two Part-annual Periods of Measurement and the Genetic Correlation between them 630
McClymont, G. L.—
See Wynne, K. N. 45
Mutation, in *Drosophila melanogaster*, Effect of Cold Treatment on 566

Nay, T., and Hayman, R. H.—
Sweat Glands in Zebu (*Bos*

PAGE

indicus L.) and European (*B. taurus* L.) Cattle. I. Size of Individual Glands, the Denseness of their Population, and their Depth below the Skin Surface .. 482

Neal-Smith, C. A.—
See Andrew, W. D. 367

Nitrogen Studies in Black Soils from Darling Downs, Queensland. I, II 169, 184

Norris, K. R., and Stone, B. F.—
Toxaphene-resistant Cattle Ticks (*Boophilus microplus* (Canestrini)) occurring in Queensland 211

Pastures, Defoliation Intensity, Effect on Regrowth 377

Pastures, Natural, Grazing Management at Trangie, N.S.W. 233

Parna, in Riverine Plain of South-eastern Australia, and Soils thereon 536

Pea Mosaic Virus, Inheritance of Immunity to 272

Pennisetum clandestinum Hochst. ex Chiov. (kikuyu grass), Induction of Flower Formation in 1

Phalaris Staggers in Sheep, Aetiology. II 333

Phalaris–Subterranean Clover Pasture Mixtures, Effect of Associate Perennial Grasses on 367

Phosphorus Deficiency, in Citrus, and Fruit Quality 261

Potassium Levels in Apricot Trees, and Brown Rot Susceptibility 516

PAGE

Potato Viruses X and Y, Effect of Malachite Green on 428

Potato Virus X, Definition of Strains present in New Zealand Potato Varieties .. 527

Pregnancy, and Birth Weight in Merino Sheep, Effect of High Air Temperature on 435

Pregnancy, and Birth Weight in Merino Sheep, Effect of Light on 440

Progesterone and Pregnant Mare Serum, Effects on the Anoestrous Ewe. I, II 591, 601

Pruning of Sultana Vines, with Constant Number of Buds per Cane, and Number of Buds per Vine Varied 401

Rams, Young Merino, Development of Testis, Epididymis, and Penis in 574

Raeside, J. I., and Lamond, D. R.—
Effects of Progesterone and Pregnant Mare Serum (PMS) Administration in the Anoestrous Ewe. I. Oestrus, Ovarian Changes, and Gonadotrophic Potency of the Pituitary Gland .. 591
II. Histological Examination of the Genital Tract .. 601

Rendel, J. M., and Sheldon, B. L.—
Effect of Cold Treatment on Mutation in *Drosophila melanogaster* 566

Rice Crop, Lowland, and Gypsum, Effects on a Saline Alkali Clay 110

PAGE

Riceman, D. S., and Jones, G. B.—
Distribution of Zinc and Copper in Seedlings of Subterranean Clover (*Trifolium subterraneum* L.) in Solution Culture 495

Riverine Plain (N.S.W.), Parna in, and Soils thereon 536

Riverine Plain (N.S.W.), Studies in Ecology of. II . . 127

Robinson, T. J.—
The Artificial Insemination of the Merino Sheep Following the Synchronization of Oestrus and Ovulation by Progesterone Injected alone and with Pregnant Mare Serum Gonadotrophin (PMS) 194

Robinson, T. J., Binet, F. E., and Doig, Alison G.—
Fat Lamb Studies in Victoria. I. An Assessment of the Relative Value of Various External Measurements for Differentiating between Various Grades of Export Lamb Carcasses . . 345

Roulston, W. J.—
Effects of some Chlorinated Hydrocarbons as Systemic Acaricides against the Cattle Tick, *Boophilus microplus* (Canestrini) . . 608

Sapsford, C. S.—
See Watson, R. H. 574

Schinckel, P. G.—
Body-weight – fleece-weight Relationships in a Strain of Merino Sheep 57

PAGE

Sclerotinia fructicola (Wint.) Rehm., and Brown Rot of Apricots. I, II 504, 516

Self-licking, by Cattle, Effect on Cattle Tick Infestations 227

Sheep, Hepatotoxic and Other Effects of Consumption of *Heliotropium europaeum* L. 281

Sheep, Merino, Artificial Insemination Following Synchronization of Oestrus and Ovulation by Progesterone Injected alone and with Pregnant Mare Serum Gonadotrophin 194

Sheep, Merino, Body-weight–fleece-weight Relationships in a Strain of 57

Sheep, Merino, Breeding Season, Gestation, and Birth Weight in, Effect of Light on 440

Sheep, Merino, Pregnancy and Birth Weight in, Effect of High Air Temperature on 435

Sheep, Merino, Selection for Economic Characters in. VII 140

Sheep, Ovine Hypocuprosis in, Copper - molybdenum - sulphate Interaction in Induction of 45

Sheep, Phalaris Staggers in, Aetiology. II 333

Sheep, Romney (New Zealand), Gene Dosage in *N*-type, Some Aspects of . . 447

Sheldon, B. L.—
Genetic Parameters Associated with Characters

PAGE

Affecting Egg Production in the Domestic Fowl. I. Heritability of Total Egg Production during the Pullet Year 625
Sheldon, B. L.—
See also Rendel, J. M. 566
Snowball, G. J.—
The Effect of Self-licking by Cattle on Infestations of Cattle Tick, *Boophilus microplus* (Canestrini) .. 227
Soil Dispersion, in Water, Time and Temperature as Factors in 554
Soil Salinity, Seasonal Changes at Tintinara, S.A. 20
Soils, Black, from Darling Downs, Queensland, Nitrogen Studies on. I, II .. 169, 184
Stephenson, S. K.—
Some Aspects of Gene Dosage in *N*-type Sheep .. 447
Stone, B. F.—
See Norris, K. R. 211
Subterranean Clover, Flowering Time in 388
Subterranean Clover–Phalaris Pasture Mixtures, Effect of Associate Perennial Grasses on 367
Subterranean Clover, Pre-emergence and Early Vegetative Growth in, Influence of Seed Size and Depth of Sowing 98
Subterranean Clover, Zinc and Copper Distribution in Seedlings in Solution Culture 495
Sulphate, Interaction with Copper and Molybdenum in

PAGE

Induction of Ovine Hypocuprosis 45
Sultana Vine, Irrigated, Chloride Status and Relation to Vine Health 414
Sultana Vine, Studies on. IV 401
Sweat Glands, in Zebu and European Cattle. I 482

Testis, Epididymis, and Penis in the Young Merino Ram, Development of 574
Thomson, A. D.—
Definition of Potato Virus X Strains present in New Zealand Potato Varieties .. 527
Studies on the Effect of Malachite Green on Potato Viruses X and Y 428
Tintinara (S.A.), Seasonal Changes in Soil Salinity at 30
Toxaphene-resistant Cattle Ticks occurring in Queensland 211
Trangie (N.S.W.), Grazing Management of Natural Pasture at 233
Trowbridge, R. F.—
See Lee, H. J. 333

Vines, Spring Frost Damage to 163

Wade, G. C.—
Investigations on Brown Rot of Apricots Caused by *Sclerotinia fructicola* (Wint.) Rehm. I. The Occurrence of Latent Infection in Fruit 504
II. Relationship of the Potassium Status of Apricot Trees to Brown Rot Susceptibility 516

PAGE

Watson, R. H., Sapsford, C. S., and McCance, I.—
Development of the Testis, Epididymis, and Penis in the Young Merino Ram .. 574

Webster, W. J.—
See Antcliff, A. J. 401

Williams, O. B.—
Studies in the Ecology of the Riverine Plain. II. Plant-soil Relationships in three Semi-arid Grasslands 127

Williams, R. F., and Gates, C. T.—
Studies in Citrus Nutrition. I. Leaf Growth and Composition 248

Woodham, R. C.—
The Chloride Status of the Irrigated Sultana Vine and its Relation to Vine Health 414

Wool Fibres, Adult Diameter, and Birthcoat of Lambs .. 152

Wool Fibres, Primary and Secondary, Reaction to Changes in Nutrition 147

PAGE

Wool Production, per Unit Area of Skin, Variation in 158

Wynne, K. N., and McClymont, G. L.—
Copper - molybdenum - sulphate Interaction in Induction of Ovine Hypocupraemia and Hypocuprosis 45

Yeates, N. T. M.—
The Effect of High Air-Temperature on Pregnancy and Birth Weight in Merino Sheep 435
The Effect of Light on the Breeding Season, Gestation, and Birth Weight of Merino Sheep 440

Yen, D. E., and Fry, P. R.—
The Inheritance of Immunity to Pea Mosaic Virus 272

Zinc, Distribution in Subterranean Clover Seedlings in Solution Culture 495

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CONTENTS

PAGE

Distribution of Zinc and Copper in Seedlings of Subterranean Clover (*Trifolium subterraneum* L.) in Solution Culture. By D. S. Riceman and G. B. Jones 495

Investigations on Brown Rot of Apricots Caused by *Sclerotinia fructicola* (Wint.) Rehm. I. The Occurrence of Latent Infection in Fruit. By G. C. Wade 504

Investigations on Brown Rot of Apricots Caused by *Sclerotinia fructicola* (Wint.) Rehm. II. The Relationship of the Potassium Status of Apricot Trees to Brown Rot Susceptibility. By G. C. Wade 516

Definition of Potato Virus X Strains Present in New Zealand Potato Varieties. By A. D. Thomson 527

Parna in the Riverine Plain of South-eastern Australia and the Soils thereon. By B. E. Butler and J. T. Hutton 536

Time and Temperature as Factors in the Dispersion of Soil Crumbs in Water. By A. V. Blackmore 554

The Effect of Cold Treatment on Mutation in *Drosophila melanogaster*. By J. M. Rendel and B. L. Sheldon 566

The Development of the Testis, Epididymis, and Penis in the Young Merino Ram. By R. H. Watson, C. S. Sapsford, and I. McCance 574

Effects of Progesterone and Pregnant Mare Serum (PMS) Administration in the Anoestrous Ewe. I. Oestrus, Ovarian Changes, and Gonadotrophic Potency of the Pituitary Gland. By J. I. Raeside and D. R. Lamond 591

Effects of Progesterone and Pregnant Mare Serum (PMS) Administration in the Anoestrous Ewe. II. Histological Examination of the Genital Tract. By J. I. Raeside and D. R. Lamond .. 601

The Effects of Some Chlorinated Hydrocarbons as Systemic Acaricides Against the Cattle Tick, *Boophilus microplus* (Canestrini). By W. J. Roulston 608

Genetic Parameters Associated with Characters Affecting Egg Production in the Domestic Fowl. I. Heritability of Total Egg Production During the Pullet Year. By B. L. Sheldon 625

Genetic Parameters Associated with Characters Affecting Egg Production in the Domestic Fowl. II. Heritability of Egg Production for Two Part-annual Periods of Measurement and the Genetic Correlation between Them. By J. A. Morris 630

Index to Volume 7 641

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